

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/31, C07K 14/40, G01N 33/50, C12Q 1/68, A61K 31/7088, 38/16, C07K 16/14	A2	(11) International Publication Number: WO 00/34481 (43) International Publication Date: 15 June 2000 (15.06.00)
(21) International Application Number: PCT/EP99/09833 (22) International Filing Date: 6 December 1999 (06.12.99) (30) Priority Data: 98204122.0 4 December 1998 (04.12.98) EP (71) Applicant (for all designated States except US): JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): CONTRERAS, Roland, Henri [BE/BE]; University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent (BE). NELISSEN, Bart [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). DE BACKER, Marianne, Denise [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). LUYTEN, Walter, Herman, Maria, Louis [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 beerse (BE). VIAENE, Jasmine, Elza [BE/BE]; University of Gent, K.L. Ledeganck Straat 35, B-9000 Gent (BE). LOGGHE, Marc, George [BE/BE]; University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent (BE). VIALARD,	Jorge, Edwards [CA/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). (74) Agent: BOULT WADE TENNANT; 27 Furnival Street, London EC4A 1PQ (GB). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(54) Title: DRUG TARGETS IN <i>CANDIDA ALBICANS</i> (57) Abstract <p>The present invention is concerned with the identification of genes or functional fragments thereof from <i>Candida albicans</i> which are critical for growth and cell division and which genes may be used as selective drug targets to treat <i>Candida albicans</i> associated infections. Novel nucleic acid sequences from <i>Candida albicans</i> are also provided and which encode the polypeptides which are critical for growth of <i>Candida albicans</i>. Methods for the identification of anti-fungal compounds which inhibit fungal or yeast growth are also contemplated.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

- 1 -

DRUG TARGETS IN *CANDIDA ALBICANS*

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides which are critical for growth of *Candida albicans*.

Opportunistic infections in immunocompromised hosts represent an increasingly common cause of mortality and morbidity. *Candida* species are among the most commonly identified fungal pathogens associated with such opportunistic infections, with *Candida albicans* being the most common species. Such fungal infections are thus problematical in, for example, AIDS populations in addition to normal healthy women where *Candida albicans* yeasts represent the most common cause of vulvovaginitis.

Although compounds do exist for treating such disorders, such as, amphotericin, these drugs are generally limited in their treatment because of their toxicity and side effects. Therefore, there exists a need for new compounds which may be used to treat *Candida* associated infections in addition to compounds which are selective in their action against *Candida albicans*.

Classical approaches for identifying anti-fungal compounds have relied almost exclusively on inhibition of fungal or yeast growth as an endpoint. Libraries of natural products, semi-synthetic, or synthetic chemicals are screened for their ability to kill or arrest growth of the target pathogen or a related nonpathogenic model organism. These tests are

- 2 -

cumbersome and provide no information about a compound's mechanism of action. The promising lead compounds that emerge from such screens must then be tested for possible host-toxicity and detailed
5 mechanism of action studies must subsequently be conducted to identify the affected molecular target.

The present inventors have now identified a range of nucleic acid sequences from *Candida albicans* which encode polypeptides which are critical for its
10 survival and growth. These sequences represent novel targets which can be incorporated into an assay to selectively identify compounds capable of inhibiting expression of such polypeptides and their potential use in alleviating diseases or conditions associated
15 with *Candida albicans* infection.

Therefore, according to a first aspect of the invention there is provided a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which
20 nucleic acid molecule comprises any of the sequences of nucleotides illustrated in any of Sequence ID Nos. 1 to 9.

Whilst the molecules defined herein have been established as being critical for growth and
25 metabolism of *Candida albicans*, for some of the molecules no apparent functionality has been assigned by virtue of the fact that no functionally related sequences in other prokaryotic or eukaryotic organism can be found in respective databases. Thus,
30 advantageously these sequences may be species specific in which case they may be used as selective targets for treatment of diseases mediated by *Candida Albicans* infection. Thus, in one aspect of the invention the nucleic acid molecules preferably
35 comprise the sequences identified in sequence ID Nos. 1, 4, 5 to 9.

- 3 -

In another aspect of the invention the sequences have been arranged functionally and of nucleotides illustrated in Sequence ID Nos. 2 or 3 are preferred and even more preferably in Sequence ID No. 2 and
5 fragments or derivatives of said nucleic acid molecules.

Letters utilised in the sequences according to the invention which are not recognisable as letters of the genetic code signify a position in the nucleic
10 acid sequence where one or more of bases A, G, C or T can occupy the nucleotide position. Representative letters used to identify the range of bases which can be used are as follows:

15	M:	A or C
	R:	A or G
	W:	A or T
	S:	C or G
	Y:	C or T
20	K:	G or T
	V:	A or C or G
	H:	A or C or T
	D:	A or G or T
	B:	C or G or T
25	N:	G or A or T or C

In one embodiment of each of the above identified aspects of the invention the nucleic acid may comprise a mRNA molecule or alternatively a DNA and preferably
30 a cDNA molecule.

Also provided by the present invention is a nucleic acid molecule capable of hybridising to the nucleic acid molecules illustrated in any of Figures 1 to 9 under high stringency conditions such as
35 antisense molecule and which conditions are generally known to those of skill in the art.

Stringency of hybridisation as used herein refers

- 4 -

to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. T_m can be approximated by the formula:

5

$$81.5^{\circ}\text{C} + 16.6(\text{Log}_{10}[\text{Na}^+] + 0.41 (\% \text{G\&C}) - 600\text{L}/\text{L})$$

wherein L is the length of the hybrids in nucleotides. T_m decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

10 The term "stringency" refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency conditions disfavour non-homologous base pairing.

"Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

25 "High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaH_2PO_4 , 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

35 "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium

- 5 -

chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄ and 1 mM EDTA, pH 7.4.

5 The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences illustrated in any of Figures 1
10 to 9.

 The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express polypeptides encoded therefrom in a suitable host which are critical for growth and
15 survival of *Candida albicans*.

 An expression vector according to the invention includes a vector having a nucleic acid according to the invention operably linked to regulatory sequences, such as promoter regions, that are capable of
20 effecting expression of said DNA fragments. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host
25 cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed,
30 transfected or infected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

35 The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression

- 6 -

of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

5 Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and
10 for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon
15 AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

 Polynucleotides according to the invention may be
20 inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

 In accordance with the present invention, a
25 defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the
30 degenerate code. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence given regarding base variations.

 The present invention also comprises within its scope proteins or polypeptides expressed by the
35 nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

- 7 -

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to the invention and preferably from 10 to approximately 120 nucleotides. In another aspect of the invention, nucleotide acid sequences are provided from 10 to 50 nucleotides. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex formation between the probe and any nucleic acid in the sample.

According to the present invention, these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesized *in situ* on the array. See Lockhart et al., Nature Biotechnology, Vol. 14, December 1996, "Expression monitoring by hybridization to high-density oligonucleotide arrays." A single array can contain more than up to more than a million different probes in discrete locations.

Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be between approximately 10 to 120 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA

- 8 -

from a cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolated the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook et al (Molecular Cloning: a Laboratory Manual, 1989).

The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{35}S , enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and may be detected using known techniques *per se*.

The polypeptide or protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are substantially homologous to said polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% amino acid homology with the polypeptides encoded by the nucleic acid molecules according to the invention.

Nucleic acids and polypeptides which are particularly preferred are those comprising the sequences of nucleotides illustrated in figures 1 to 3 and polypeptides illustrated in figures 14 to 16. However, a particularly preferred nucleic acid comprises the sequences of nucleotides illustrated in Figures 2 and/or 3, and their corresponding amino acid sequences identified in Figures 15 and 16.

Nucleotide sequences according to the invention

- 9 -

are particularly advantageous as selective therapeutic targets for treating *Candida albicans* associated infections. For example, an antisense nucleic acid capable of binding to the nucleic acid sequence
5 illustrated in any of Figures 1 to 9 may be used to selectively inhibit expression of the corresponding polypeptides, leading to impaired growth of the *Candida albicans* with reductions of associated illnesses or diseases.

10 The nucleic acid molecule or the polypeptide according to the invention may be used as a medicament, or in the preparation of a medicament, for treating diseases or conditions associated with *Candida albicans* infection.

15 Advantageously, the nucleic acid molecule or the polypeptide according to the invention may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

20 The present invention is further directed to inhibiting expression of nucleic acids according to the invention *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation
25 of antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion or the mature protein sequence, which encodes for the protein of the present invention, is used to design an antisense RNA
30 oligonucleotide of from 10 to 50 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee et al. Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241:456 (1988);
35 and Dervan et al., Science, 251: 1360 (1991), thereby preventing transcription and the production of the

- 10 -

corresponding protein. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the corresponding protein (antisense - Okano, J.

5 Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by
10 techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse, with the polypeptide according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be
15 prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497.

Antibodies according to the invention may also be used in a method of detecting for the presence of a
20 polypeptide according to the invention, which method comprises reacting the antibody with a sample and identifying any protein bound to said antibody. A kit may also be provided for performing said method which comprises an antibody according to the invention and
25 means for reacting the antibody with said sample.

Proteins which interact with the polypeptide of the invention may be identified by investigating protein-protein interactions using the two-hybrid vector system first proposed by Chien *et al.* (1991).

30 This technique is based on functional reconstitution *in vivo* of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under
35 the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA

- 11 -

sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

15 An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a reporter molecule in a GAL4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as β -galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

Further provided by the present invention is one

- 12 -

or more *Candida albicans* cells comprising an induced mutation in the DNA sequence encoding the polypeptide according to the invention.

5 A further aspect of the invention provides a method of identifying compounds which selectively inhibit or interfere with the expression, the functionality of polypeptides expressed from the nucleotides sequences illustrated in any of Figures 1 to 9 or the metabolic pathways in which these
10 polypeptides are involved and which are critical for growth and survival of *Candida albicans*, which method comprises (a) contacting a compound to be tested with one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to the invention
15 which mutation results in overexpression or underexpression of said polypeptides in addition to one or more wild type *Candida* cells, (b) monitoring the growth and/or activity of said mutated cell compared to said wild type wherein differential growth
20 or activity of said one or more mutated *Candida* cells provides an indication of selective action of said compound on said polypeptide or another polypeptide in the same or a parallel pathway.

Compounds identifiable or identified using the
25 method according to the invention, may advantageously be used as a medicament, or in the preparation of a medicament to treat diseases or conditions associated with *Candida albicans* infection. These compounds may also advantageously be included in a pharmaceutical
30 composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

A further aspect of the invention provides a method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are
35 critical for growth or survival, which method comprises (a) preparing a cDNA or genomic library from

- 13 -

said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library, (b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. Preferably, the cell or organism may be any yeast or filamentous fungus, such as, for example, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

A further aspect of the invention provides a pharmaceutical composition comprising any of a compound, an antisense molecule or an antibody according to the invention together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The antisense molecules or indeed the compounds identified as agonists or antagonists of the nucleic acids or polypeptides according to the invention may be used in the form of a pharmaceutical composition, which may be prepared according to procedures well known in the art. Preferred compositions include a pharmaceutically acceptable vehicle or diluent or excipient, such as for example, a physiological saline solution. Other pharmaceutically acceptable carriers including other non-toxic salts, sterile water or the like may also be used. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for subsequent administration. Incorporation of the polypeptides of the invention into a solid or semi-solid biologically compatible matrix may be carried out which can be implanted into tissues requiring treatment.

- 14 -

The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like.

5 Pharmaceutically acceptable excipients which permit sustained or delayed release following administration may also be included.

The polypeptides, the nucleic acid molecules or compounds according to the invention may be
10 administered orally. In this embodiment they may be encapsulated and combined with suitable carriers in solid dosage forms which would be well known to those skilled in the art.

As would be well known to those of skill in the
15 art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent upon the particular route of administration to be used. The amount of the composition actually
20 administered will, however, be determined by a medical practitioner, based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the composition to be administered, the age, weight, and response of the individual patient
25 and the chosen route of administration.

The present invention may be more clearly understood with reference to the accompanying example, which is purely exemplary, with reference to the accompanying drawings, wherein

30

Figures 1 and 2: are nucleotide sequences isolated from *Candida albicans* and which have an identified function based on sequence homology with proteins from other organisms and which
35 sequences are not present in the public domain.

- 15 -

- 5 Figures 3 : is a nucleotide sequence isolated from *Candida albicans* and which has an identified function based on sequence homology with proteins from other organisms and which sequence is partially present in the public domain.
- 10 Figures 4 : is a nucleotide sequence of previously unknown function isolated from *Candida albicans* and which is partially present in the public domain.
- 15 Figures 5 to 9 : are nucleotide sequences of previously unknown function isolated from *Candida albicans*.
- 20 Figure 10 : is a diagrammatic representation of plasmid pGAL1PNiST-1.
- Figure 11 : is a nucleotide sequence of plasmid pGAL1PNiST-1 of Figure 10.
- 25 Figure 12 : is a diagrammatic representation of plasmid pGAL1PSiST-1.
- Figure 13 : is a nucleotide sequence of plasmid pGAL1PSiST-1 of Figure 12.
- 30 Figures 14 to 20: are amino acid sequences of the appropriately corresponding DNA sequences illustrated in Figures 1 to 9 with reference to Table 1.
- 35 Figures 21 to 27: are growth curves of *Candida albicans* strains showing antisense

- 16 -

induced reduction in growth.

Figures 28 to 31: are growth curves of *Candida albicans* strains including knock-outs in the relevant gene identified.

Example 1

Identification of novel drug targets in *C. albicans* by anti-sense and disruptive integration

The principle of the approach is based on the fact that when a particular *C. albicans* mRNA is inhibited by producing the complementary anti-sense RNA, the corresponding protein will decrease. If this protein is critical for growth or survival, the cell producing the anti-sense RNA will grow more slowly or will die.

Since anti-sense inhibition occurs at mRNA level, the gene copy number is irrelevant, thus allowing applications of the strategy even in diploid organisms.

Anti-sense RNA is endogenously produced from an integrative or episomal plasmid with an inducible promoter; induction of the promoter leads to the production of an RNA encoded by the insert of the plasmid. This insert will differ from one plasmid to another in the library. The inserts will be derived from genomic DNA fragments or from cDNA to cover to the extent possible the entire genome.

The vector is a proprietary vector allowing integration by homologous recombination at either the homologous insert or promoter sequence in the *Candida* genome. After introducing plasmids from cDNA or genomic libraries into *C. albicans*, transformants are screened for impaired growth after promoter (& thus anti-sense) induction in the presence of lithium

- 17 -

acetate. Lithium acetate prolongs the G1 phase and thus allows anti-sense to act during a prolonged period of time during the cell cycle. Transformants which show impaired growth in both induced and non-induced media, thus showing a growth defect due to integrative disruption, are selected as well.

Transformants showing impaired growth are supposed to contain plasmids which produce anti-sense RNA to mRNAs critical for growth or survival. Growth is monitored by measuring growth-curves over a period of time in a device (Bioscreen Analyzer, Labsystems) which allows simultaneous measurement of growth-curves of 200 transformants.

Subsequently plasmids can be recovered from the transformants and the sequence of their inserts determined, thus revealing which mRNA they inhibit. In order to be able to recover the genomic or cDNA insert which has integrated into the *Candida* genome, genomic DNA is isolated, cut with an enzyme which cuts only once into the library vector (and estimated approx. every 4096 bp in the genome) and religated. PCR with primers flanking the insert will yield (partial) genomic or cDNA inserts as PCR fragments which can directly be sequenced. This PCR analysis (on ligation reaction) will also show us how many integrations occurred. Alternatively the ligation reaction is transformed to *E. coli* and PCR analysis is performed on colonies or on plasmid DNA derived thereof.

This method is employed for a genome-wide search for novel *C. albicans* genes which are important for growth or survival.

Materials & Methods

Construction of pGallPNiST-1

The backbone of the pGALLPNiST-1 vector (integrative anti-sense *SfiI*-*NotI* vector) is

- 18 -

pGEM11Zf(+) (Promega Inc.). First, the CaMAL2
EcoRI/SalI promoter fragment from pDBV50 (D.H. Brown
et al. 1996) was ligated into EcoRI/SalI-opened
pGEM11Zf(+) resulting in the intermediate construct
5 pGEMMAL2P-1. Into the latter (MscI/CIP) the CaURA3
selection marker was cloned as a Eco47III/XmnI
fragment derived from pRM2. The resulting pGEMMAL2P-2
vector was NotI/HindIII opened in order to accept the
NotI-stuffer-SfiI cassette from pPCK1NiSCYCT-1
10 (EagI/HindIII fragment): pMAL2PNiST-1. Finally, the
plasmid pGAL1PNiST-1 was constructed by exchanging the
SalI/Ecl136II MAL2 promoter in pMAL2PNiST-1 by the
XhoI/SmaI GAL1 promoter fragment derived from
pRM2GAL1P.

15

Construction of pGallPSiST-1

The vector pGAL1PSiST-1 was created for cloning
the small genomic DNA fragments (flanked by SfiI
sites) behind the GAL1 promoter. The only difference
20 with pGAL1PNiST-1 is that the hIFN β (stuffer fragment)
insert fragment in pGAL1PSiST-1 is flanked by two SfiI
sites instead of a SfiI and a NotI site as in
pGAL1PNiST-1. To construct pGAL1PSiST-1 the EcoRI-
HindIII fragment, containing hIFN β flanked by a SfiI
25 and a NotI site, of pMAL2pHiET-3 (unpublished) was
exchanged by the EcoRI-HindIII fragment, containing
hIFN β flanked by two SfiI sites, from YCp50S-S (an *E.*
coli / *S. cerevisiae* shuttle vector derived from the
plasmid YCp50, which is deposited in the ATCC
30 collection (number 37419; Thrash et al., 1985); an
EcoRI-HindIII fragment, containing the gene hIFN β ,
which is flanked by two SfiI sites, was inserted in
YCp50, creating YCp50S-S), resulting into plasmid
pMAL2PSiST-1. The MAL2 promoter from pMAL2PSiST-1 (by
35 a NaeI-balI digest) was further replaced by the GAL1
promoter from pGAL1PNiST-1 (via a XhoI-FSPI digest),

- 19 -

creating the vector pGAL1PSiST-1.

***Candida albicans* genomic library**

*** Preparation of the genomic DNA fragments**

5 A *Candida albicans* genomic DNA library with small DNA fragments (400 to 1,000 bp) was prepared. Genomic DNA of *Candida albicans* B2630 was isolated following a modified protocol of Blin and Stafford (1976). The quality of the isolated genomic DNA was checked by gel
10 electrophoresis. Undigested DNA was located on the gel above the marker band of 26,282 bp. A little smear, caused by fragmentation of the DNA, was present. To obtain enrichment for genomic DNA fragments of the desired size, the genomic DNA was partially digested.
15 Several restriction enzymes (*AluI*, *HaeIII* and *RsaI*; all creating blunt ends) were tried out. The appropriate digest conditions have been determined by titration of the enzyme. Enrichment of small DNA fragments was obtained with 70 units of *AluI* on 10 µg
20 of genomic DNA for 20 min. T4 DNA polymerase (Boehringer) and dNTPs (Boehringer) were added to polish the DNA ends. After extraction with phenol-chloroform the digest was size-fractionated on an agarose gel. The genomic DNA fragments with a length
25 of 500 to 1,250 bp were eluted from the gel by centrifugal filtration (Zhu et al., 1985). *SfiI* adaptors (5' GTTGGCCTTTT) or (5' AGGCCAAC) were attached to the DNA ends (blunt) to facilitate cloning of the fragments into the vector. Therefore, a 8-mer
30 and 11-mer oligonucleotide (comprising the *SfiI* site) were kinased and annealed. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The DNA fragments of 400 to 1150 bp were eluted from the
35 gel by centrifugal filtration.

*** Preparation of the pGAL1PSiST-1 vector fragment**

- 20 -

The small genomic DNA fragments were cloned after the GAL1 promoter in the vector pGAL1PSiST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with *SfiI* and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu et al., 1985).
5 Ligation with a control DNA fragment, flanked by *SfiI* sites, was performed as a control. The ligation mix was electroporated to MC1061 *E. coli* cells. Plasmid DNA of 24 clones was analyzed. In all cases the
10 control fragment was inserted in the pGAL1PSiST-1 vector fragment.

** Upscaling*

All genomic DNA fragments (450 ng) were ligated into the pGAL1PSiST-1 vector (20 ng). After
15 electroporation at 2500V, 40µF circa 400,000 clones were obtained. These clones were pooled into three groups and stored as glycerol slants. Also Qiagen-purified DNA was prepared from these clones. A clone
20 analysis showed an average insert length of 600 bp and a percentage of 91 for clones with an insert. The size of the library corresponds to 5 times the diploid genome. The genomic DNA inserts are sense or anti-sense orientated in the vector.

25 ***Candida albicans* cDNA library**

Total RNA was extracted from *Candida albicans* B2630 grown on respectively minimal (SD) and rich (YPD) medium as described by Chirgwin et al. in Sambrook et al 1996. mRNA was prepared from total RNA
30 using the Invitrogen Fast Track procedure.

First strand cDNA is synthesised with the Superscript Reverse Transcriptase (BRL) and with an oligo dT-*NotI* Primer adapter. After second strand synthesis, cDNA is polished with Klenow enzyme and
35 purified over a Sephacryl S-400 spun column. Phosphorylated *SfiI* adapters are then ligated to the

- 21 -

cDNA, followed by digestion with the *NotI* restriction enzyme. The *SfiI/NotI* cDNA is then purified and sized on a Biogel column A150M.

First fraction contains approximately 38,720 clones by transformation, the second fraction only 1540 clones. Clone analysis:
Fr. I: 22/24 inserts, 16 ³ 1000 bp, 4 ³ 2000 bp, average size: 1500 bp.
Fr. II: 9/12 inserts, 3 ³ 1000 bp, average size: 960 bp cDNA was ligated in a *NotI/SfiI* opened pGAL1PNiST-1 vector (anti-sense)

Candida transformation

The host strain used for transformation is a *C. albicans* *ura3* mutant, CAI-4, which contains a deletion in orotidine-5'-phosphate decarboxylase and was obtained from William Fonzi, Georgetown University (Fonzi and Irwin). CAI-4 was transformed with the above described cDNA library or genomic library using the *Pichia* spheroplast module (Invitrogen). Resulting transformants were plated on minimal medium supplemented with glucose (SD, 0.67% or 1.34% Yeast Nitrogen base w/o amino acids + 2% glucose) plates and incubated for 2-3 days at 30°C.

25

Screening for mutants

Starter cultures were set up by inoculating each colony in 1 ml SD medium and incubating overnight at 30°C and 300 rpm. Cell densities were determined using a Coulter counter (Coulter Z1; Coulter electronics limited). 250.000 cells/ml were inoculated in 1 ml SD medium and cultures were incubated for 24 hours at 30°C and 300 rpm. Cultures were washed in minimal medium without glucose (S) and the pellet resuspended in 650 µl S medium. 8 µl of this culture is used for inoculating 400 µl cultures in a Honeywell-100 plate

35

- 22 -

(Bioscreen analyzer; Labsystems). Each transformant was grown during three days in S medium containing LiAc; pH 6.0, with 2% glucose/2% maltose or 2% galactose/2% maltose respectively while shaking every 3 minutes for 20 seconds. Optical densities were measured every hour during three consecutive days and growth curves were generated (Bioscreen analyzer; Labsystems).

Growth curves of transformants grown in respectively anti-sense non-inducing (glucose/maltose) and inducing (galactose/maltose) medium are compared and those transformants showing impaired growth upon anti-sense induction are selected for further analysis. Transformants showing impaired growth by virtue of integration into a critical gene are also selected.

Isolation of genomic or cDNA inserts

Putatively interesting transformants are grown in 1.5 ml SD overnight and genomic DNA is isolated using the Nucleon MI/Yeast kit (Clontech). Concentration of genomic DNA is estimated by analyzing a sample on an agarose gel.

20 ng of genomic DNA is digested for three hours with an enzyme that cuts uniquely in the library vector (SacI for the genomic library; PstI for the cDNA library) and treated with RNase. Samples are phenol/chloroform extracted and precipitated using NaOAc/ethanol.

The resulting pellet is resuspended in 500 μ l ligation mixture (1 x ligation buffer and 4 units of T4 DNA ligase; both from Boehringer) and incubated overnight at 16°C.

After denaturation (20 min 65°C), purification (phenol/chloroform extraction) and precipitation (NaOAc/ethanol) the pellet is resuspended in 10 μ l MilliQ (Millipore) water.

- 23 -

PCR analysis

Inverse PCR is performed on 1 μ l of the precipitated ligation reaction using library⁺ vector specific primers (oligo23 5' TGC-AGC-TCG-ACC-TCG-ACT-G 3' and oligo25 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' for the genomic library; 3pGALNistPCR primer :5'TGAGCAGCTCGCCGTCGCGC 3' and 5pGALNistPCR primer: 5'GAGTTATACCCTGCAGCTCGAC 3' for the cDNA library; both from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 57 °C, and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 μ M of each primer, 3 mM MgCl₂ (Perkin Elmer Cetus) and 200 μ M dNTPs (Perkin Elmer Cetus). PCR was performed in a Robocycler (Stratagene).

Sequence determination

Resulting PCR products were purified using PCR purification kit (Qiagen) and were quantified by comparison of band intensity on EtBr stained agarose gel with the intensity of DNA marker bands. The amount of PCR product (expressed in ng) used in the sequencing reaction is calculated as the length of the PCR product in basepairs divided by 10. Sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the instructions of the manufacturer (PE Applied Biosystems, Foster City, CA) except for the following modifications.

The total reaction volume was reduced to 15 μ l. Reaction volume of individual reagents were changed accordingly. 6.0 μ l Terminator Ready Reaction Mix was replaced by a mixture of 3.0 μ l Terminator Ready Reaction Mix + 3.0 μ l Half Term (GENPAK Limited,

- 24 -

Brighton, UK). After cycle sequencing, reaction mixtures were purified over Sephadex G50 columns prepared on Multiscreen HV opaque microtiter-plates (Millipore, Molsheim, Fr) and were dried in a speedVac. Reaction products were resuspended in 3 μ l loading buffer. Following denaturation for 2 min at 95°C, 1 μ l of sample was applied on a 5% Long Ranger Gel (36 cm well-to-read) prepared from Singel Packs according to the supplier's instructions (FMC BioProducts, Rockland, ME). Samples were run for 7 hours 2X run on a ABI 377XL DNA sequencer. Data collection version 2.0 and Sequence analysis version 3.0 (for basecalling) software packages are from PE Applied Biosystems. Resulting sequence text files were copied onto a server for further analysis.

Sequence analysis

Nucleotide sequences were imported in the VectorNTI software package (InforMax Inc, North Bethesda, MD, USA), and the vector and insert regions of the sequences were identified. Sequence similarity searches against public and commercial sequence databases were performed with the BLAST software package (Altschul et al., 1990) version 1.4. Both the original nucleotide sequence and the six-frame conceptual translations of the insert region were used as query sequences. The used public databases were the EMBL nucleotide sequence database (Stoesser et al., 1998), the SWISS-PROT protein sequence database and its supplement TrEMBL (Bairoch and Apweiler, 1998), and the ALCES *Candida albicans* sequence database (Stanford University, University of Minnesota). The commercial sequence databases used were the LifeSeq® human and PathoSeq® microbial genomic databases (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA), and the GENESEQ patent sequence database (Derwent, London,

- 25 -

UK). Three major results were obtained on the basis of the sequence similarity searches: function, novelty, and specificity. A putative function was deduced on the basis of the similarity with sequences with a known function, the novelty was based on the absence or presence of the sequences in public databases, and the specificity was based on the similarity with vertebrate homologues.

10 **Methods**

Blastx of the nucleic acid sequences against the appropriate protein databases: Swiss-Prot for clones of which the complete sequence is present in the public domain, and paorfp (PathoSeq™) for clones of which the complete sequences is not present in the public domain.

The protein to which the translated nucleic acid sequence corresponds to is used as a starting point. The differences between this protein and our translated nucleic acid sequences are marked with a double line and annotated above the protein sequence. The following symbols are used:

a one-letter amino acid code or the ambiguity code X is used if our translated nucleic acid sequence has another amino acid on a certain position, the stop codon sign * is used if our translated nucleic acid sequence has a stop codon on a certain position,

The letters fs (frame shift) are used if a frame shift occurs in our translated nucleic acid sequence, and another reading frame is used,

the words ambiguity or ambiguities are used if a part of our translated nucleic acid sequence is present in the proteins, but not visible in the alignments of the blast results,

The phrase "missing sequence" is used if the translated nucleic acid sequence does not comprise

- 26 -

that part of the protein.

Blastx: compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database.

5

Gene Knock-outs

To verify that the growth effect was due to the interference with the identified gene and to support the specificity of the antisense effect, single allele knock-outs were made in the identified genes (Figures 10 28 to 31) using the URA-blaster method (Fonzi and Irwin 1993).

Screening for compounds modulating expression of polypeptides critical for growth and survival of *C. albicans*

The method proposed is based on observations (Sandbaken et al., 1990; Hinnebusch and Liebman 1991; Ribogene PCT WO 95/11969, 1995) suggesting that 20 underexpression or overexpression of any component of a process (e.g. translation) could lead to altered sensitivity to an inhibitor of a relevant step in that process. Such an inhibitor should be more potent against a cell limited by a deficiency in the 25 macromolecule catalyzing that step and/or less potent against a cell containing an excess of that macromolecule, as compared to the wild type (WT) cell.

Mutant yeast strains, for example, have shown that some steps of translation are sensitive to the 30 stoichiometry of macromolecules involved. (Sandbaken et al. 1996). Such strains are more sensitive to compounds which specifically perturb translation (by acting on a component that participates in translation) but are equally sensitive to compounds 35 with other mechanisms of action.

This method thus not only provides a means to

- 27 -

identify whether a test compound perturbs a certain process but also an indication of the site at which it exerts its effect. The component which is present in altered form or amount in a cell whose growth is
5 affected by a test compound is potentially the site of action of the test compound.

The assay to be set up involves measurement of growth of an isogenic strain which has been modified only in a certain specific allele, relative to a wild
10 type (WT) *C. albicans* strain, in the presence of R-compounds. Strains can be ones in which the expression of a specific essential protein is impaired upon induction of anti-sense or strains which carry disruptions in an essential gene. An *in silico*
15 approach to finding novel essential genes in *C. albicans* will be performed. A number of essential genes identified in this way will be disrupted (in one allele) and the resulting strains can be used for comparative growth screening.

20

Assay for High Throughput screening for drugs

35 μ l minimal medium (S medium + 2% galactose + 2% maltose) is transferred in a transparent flat-bottomed 96 well plate using an automated pipetting
25 system (Multidrop, Labsystems). A 96-channel pipettor (Hydra, Robbins Scientific) transfers 2.5 μ l of R-compound at 10^{-3} M in DMSO from a stock plate into the assay plate.

The selected *C. albicans* strains (mutant and
30 parent (CAI-4) strain) are stored as glycerol stocks (15%) at -70°C . The strains are streaked out on selective plates (SD medium) and incubated for two days at 30°C . For the parent strain, CAI-4, the medium is always supplemented with 20 $\mu\text{g/ml}$ uridine. A single
35 colony is scooped up and resuspended in 1 ml minimal medium (S medium + 2% galactose + 2% maltose). Cells

- 28 -

- are incubated at 30°C for 8 hours while shaking at 250 rpm. A 10 ml culture is inoculated at 250.000 cells/ml. Cultures are incubated at 30°C for 24 hours while shaking at 250 rpm. Cells are counted in
- 5 Coulter counter and the final culture (S medium + 2% galactose + 2% maltose) is inoculated at 20.000 to 50.000 cells/ml. Cultures are grown at 30°C while shaking at 250 rpm until a final OD of 0.24 (+/- 0.04) 600nm is reached.
- 10 200 µl of this yeast suspension is added to all wells of MW96 plates containing R-compounds in a 450 (or 250) µl total volume. MW96 plates are incubated (static) at 30°C for 48 hours.
- Optical densities are measured after 48 hours.
- 15 Test growth is expressed as a percentage of positive control growth for both mutant (x) and wild type (y) strains. The ratio (x/y) of these derived variables is calculated.

- 29 -

References

- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and
D.J. Lipman, 1990, Basic Local Alignment Search Tool,
5 J. Mol. Biol. 215: 403-410.
- Thrash C., Bankier A. T., Barrell B. G. and Sternglanz
R. (1985) *Proc. Natl. Acad. Sci. USA* 82: 4374-4378.
- 10 Blin N. and Stafford D. W. (1976) *Nucleic Acids Res.*
3: 2303-2308.
- Zhu J., Kempnaers W., Van Der Straeten D., Contreras
R., and Fiers W. (1985) *Bio/Technology* 3: 1014-1016.
- 15 Sambrook J., Fritsch E.F. and Maniatis T. (1989)
Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold
Spring Harbor Laboratory, Cold Spring Harbor, NY.
- 20 Fonzi W. and Irwin H. (1993) *Genetics* 134:717-728.
Altschul S.F., Gish W., Miller W., Myers E.W., Lipman
D.J. (1990) *J. Mol. Biol.* 215(3):403-410.
- Bairoch A. and Apweiler R. (1998) *Nucleic Acids Res.*
25 26(1):38-42.
- Stoesser G., Moseley M.A., Sleep J., McGowran M.,
Garcia-Pastor M., Sterk P. (1998) *Nucleic Acids Res.*
26(1):8-15.
- 30 Chien et al., (1991) *Proc. Natl. Acad. Sci USA* 88,
9578-9582.
- Sandbaken M.G. Lupisella J.A., DiDomenico B. and
35 Chakraborty K., *J. Biol. Chem.* 265:15838-15844, 1990.

- 30 -

Hinnebusch A.G. and Liebman S.W., in : The Molecular Biology of the Yeast *Saccharomyces*, Broach J.R., Pringle J.R. and Jones E.W., eds., CSH Laboratory Press; NY 1991.

5

Patent application RiboGene Inc., PCT WO 95./11969, 1995.

10

Brown, D.H. et al., Molecular General Genetics 251 (1) 75-80, 1996.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410.

15

Arndt, G.M., D. Atkins, M. Patrikakis, and J.G. Izant 1995. Gene regulation by antisense RNA in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. 248: 293-300.

20

Atkins, D., and W.L. Gerlach. 1994. Artificial ribozyme and antisense gene expression in *S. cerevisiae*. Antisense research and development 4:109-117.

25

Atkins, D., G.M. Arndt, and J.G. Izant 1994. Antisense gene expression in yeast. Biol. Chem. Hoppe-Seyler. 375:721-729.

30

Atkins, D., M. Patrikakis, J.G. Izant 1995. The *ade6* gene of the fission yeast as a target for antisense and ribozyme RNA-mediated suppression. Antisense Research & Development. 5(4):295-305.

35

Bairoch, A., and R. Apweiler. 1998. The SWISS-PROT protein sequence data bank and its supplement TrEMBL in 1998. Nucleic Acids Res. 26: 38-42.

- 31 -

- Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, C. Cullin. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Research*. 21(14):3329-30.
- 5 Blin, N., and D.W. Stafford. 1976. *Nucleic Acids Res.* 3: 2303-2308.
- Dujon, B. 1998. European Functional Analysis Network (EUROFAN) and the functional analysis of the *Saccharomyces cerevisiae* genome. *Electrophoresis*. 19:617-624.
- 10
- Del Rosario, M., J.C. Stephans, J. Zakel, J. Escobedo, and K. Giese. 1996. Positive selection system to screen for inhibitors of human immunodeficiency virus-1 transcription. *Nature Biotechnology*. 14:1592-1596.
- 15
- Fairhead, C., A. Thierry, F. Denis, M. Eck, and B. Dujon. 1998. "Mass-murder" of ORFs from three regions of chromosome XI from *Saccharomyces cerevisiae*. *Gene*. 223:33-46.
- 20
- Ferbeyre, G., J. Bratty, H. Chen, R. Cedergren. 1996. Cell cycle arrest promotes trans-hammerhead ribozyme action in yeast. *Journal of Biological Chemistry*. 271(32):19318-23.
- 25
- Fleischmann, R.D., M.D. Adams, O. White, R.A. Clayton, E.F. Kirkness, A.R. Kerlavage, C.J. Bult, J.F. Tomb, B.A. Dougherty, J.M. Merrick. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae*. *Science*. 269: 496-512
- 30
- Fonzi, W.A., and M.Y. Irwin. 1993. Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134:717-728.
- 35

- 32 -

- Fraser, C.M., J.D. Gocayne, O. White, M.D. Adams, R.A. Clayton, R.D. Fleischmann, C.J. Bult, A.R. Kervalage, G. Sutton, J.M. Kelley. 1995. The minimal gene complement of Mycoplasma genitalium. Science 270:397-403.
- Giese, K. 1997. Method and construct for screening for inhibitors of transcriptional activation. International patent application WO 97/10360.
- Hahn, S. Guarente L. 1988. Yeast HAP2 and HAP3: transcriptional activators in a heteromeric complex. Science. 240(4850):317-21
- Heid, C.A., J. Stevens, K.J. Livak, and P.M. Williams. 1996. Real time quantitative PCR. Genome Methods 6:986-994.
- Jayaram, M., A. Sutton, J.R. Broach. 1985. Properties of REP3: a cis-acting locus required for stable propagation of the Saccharomyces cerevisiae plasmid 2 microns circle. Molecular & Cellular Biology. 5(9):2466-75.
- Jeong, S.W., W.H. Lang, R.H. Reeder. 1996. The yeast transcription terminator for RNA polymerase I is designed to prevent polymerase slippage. Journal of Biological Chemistry. 271(27):16104-10.
- Johnson, F.B., D.A. Sinclair, and L. Guarente. 1999. Molecular Biology of aging. Cell. 96:291-302.
- Leuker, C.E., A. Sonneborn, S. Delbruck, J.F. Ernst. 1997. Sequence and promoter regulation of the PCK1 gene encoding phosphoenolpyruvate carboxykinase of the fungal pathogen Candida albicans. Gene. 192(2):235-40.

- 33 -

- Lie, Y.S., and C.J. Petropoulos. 1998. Advances in quantitative PCR technology: 5' nuclease assays. *Current Opinion in Biotechnology* 9:43-48.
- 5 Livak, K.J., S.J. Flood, J. Marmaro, W. Giusti, K. Deetz. 1995. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *Genome Research*. 4(6):357-62.
- 10 Mandart, E. 1998. Effects of mutations in the *Saccharomyces cerevisiae* RNA14 gene on the abundance and polyadenylation of its transcripts. *Mol. Gen. Genet.* 258:16-25.
- 15 McMahon, S.B., H.A. Van Buskirk, K.A. Dugan, T.D. Copeland, and M.D. Cole. 1998. The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell*. 94:363-74.
- 20 Murray, J.A.H., M. Scarpa, N. Rossi, G. Cesareni. 1987. Antagonistic controls regulate copy number of the yeast 2 μ plasmid. *EMBO J.* 6:4205-4212.
- 25 Nasr, F., A. Bécam, P.P. Slonimski, and C.J. Herbert. 1994. YBR1012, an essential gene from *S. cerevisiae*: construction of an RNA-antisense conditional allele and isolation of a multicopy suppressor. *CR Acad. Sci. Paris*. 317:607-613
- 30 Nasr, F., A. Bécam, S.C. Brown, D. De Nay. P.P. Slonimski, and C.J. Herbert. 1995. Artificial antisense regulation of YBR1012 an essential gene from *S. cerevisiae* which is important for progression through G1/S. *Mol. Gen. Genet.* 249:51-57.
- 35

- 34 -

- Nomura, T., N. Fujita, A. Ishihama. 1985. Promoter selectivity of E. coli RNA polymerase: analysis of the promoter system of convergently-transcribed dnaQ-rnh genes. Nucleic Acids Research. 13(21):7647-61.
- 5 Orlando, C., P. Pinzani, and M. Pazzagli. 1998. Developments in quantitative PCR. Clin. Chem. Lab. Med. 36(5):255-269.
- 10 Pla, J., C. Gil, F. Monteoliva, M. Sanchez, and C. Nombela. 1996. Understanding Candida albicans at the molecular level. Yeast. 12:1677-1702.
- 15 Reeder, R.H. and W.H. Lang. 1997. Terminating transcription in eukaryotes: lessons learned from RNA polymerase I. Trends in Biochemical Sciences. 22(12):473-7, 1997
- 20 Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- 25 Sinclair, D.A., L. Guarente. 1997. Extrachromosomal rDNA circles--a cause of aging in yeast. Cell. 91(7):1033-42.
- 30 Smith, V., D. Botstein, and P. O. Brown. 1995. Genetic footprinting: A genomic strategy for determining a gene's function given its sequence. Proc. Natl. Acad. Sci. USA. 92:6479-6483.
- 35 Stoesser, G., Moseley M.A., Sleep J., McGowran M., Garcia-Pastor M., Sterk P. 1998. Nucleic Acids Res. 26(1):8-15.
- Thompson-Jager, S. Domdey H. 1990. The intron of the yeast actin gene contains the promoter for an

- 35 -

antisense RNA. Current Genetics. 17(3):269-73.

Thompson, J.R., E. Register, J. Curotto, M. Kurtz, and
R. Kelly. 1998. An improved protocol for the
5 preparation of yeast cells for transformation by
electroporation. Yeast. 14:565-571.

Thrash, C., A.T. Bankier, B.G. Barrell, and R.
Sternglanz. 1985. Proc. Natl. Acad. Sci. USA 82: 4374-
10 4378.

Van Duin, M., J. van Den Tol, J.H. Hoeijmakers, D.
Bootsma, I.P. Rupp, P. Reynolds, L. Prakash, and S.
Prakash. 1989. Conserved pattern of antisense
15 overlapping transcription in the homologous human
ERCC-1 and yeast RAD10 DNA repair gene regions.
Molecular & Cellular Biology. 9(4):1794-8.

Wach, A., A. Brachat, R. Pohlmann, P. Philippsen.
20 1994. New heterologous modules for classical or PCR-
based gene disruptions in Saccharomyces cerevisiae.
Yeast. 10(13):1793-808.

Wilson, R.B., D. Davis, A.P. Mitchell. 1999. Rapid
25 hypothesis testing with Candida albicans through gene
disruption with short homology regions. Journal of
Bacteriology. 181(6):1868-74.

Zhu, J., W. Kempenaers, D. Van der Straeten, R.
30 Contreras, and W. Fiers. 1985. A method for fast and
pure DNA elution from agarose gels by centrifugal
filtration. Bio/Technology. 3: 1014-1016.

- 36 -

Table 1

Seq ID No.	Figure No.	Clone	Function
1	1	382c_cp	-
2	2	392c_cp	TUF1
3	3	-	RAD53
4	4	417c_cpG2L	-
5	5	323c_af	-
6	6	322c_cp ¹	-
7	7	26g3	-
8	8	409c_cp	-
9	9	382c_cpG1L2	-
10	14	382c_cp (prt)	-
11	15	392c_cp (prt)	TUF1
12	16		RAD53
13	17	325c_af (prt) ²	-
14	18	322c_cp (prt) ²	-
15	19	26g3 (prt)	-
16	20	417c_cp 92L (prt)	-

1. 322c-cp is a member of the UPF0057 protein family. It contains potential transmembrane regions (6-23aa; 30-53aa) and could be low temperature or salt-stress inducible.
2. 325c-af shows similarity to IMP4 yeast and related proteins and it might be involved in rRNA processing in *Candida albicans* in a similar way to IMP4.

- 37 -

Claims

1. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 to 9.
2. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 to 3.
3. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 or 2 and fragments or derivatives of said nucleic acid molecules.
4. A nucleic acid molecule according to any of claims 1 to 3 which is mRNA.
5. A nucleic acid molecule according to any of claims 1 to 3 which is DNA.
6. A nucleic acid molecule according to claim 5 which is cDNA.
7. A nucleic acid molecule capable of hybridising to the molecules according to any of claims 1 to 6 or the sequences illustrated in any of Seq ID Nos 1 to 9 under high stringency conditions.
8. An antisense molecule comprising a nucleic

- 38 -

acid molecule capable of hybridising to the molecules according to any of claims 1 to 6 or the sequences illustrated in any of Seq ID Nos 1 to 9.

5 9. Cells containing a nucleic acid molecule according to any of claims 1 to 8, wherein said cells are bacterial or eukaryotic.

10 10. A polypeptide encoded by the nucleic acid molecule according to any of claims 1 to 7 or the sequences illustrated in any of Seq ID Nos 1 to 9.

15 11. A polypeptide having any of amino acid sequences illustrated in any of Seq ID Nos 14 to 20.

 12. A recombinant DNA construct comprising a nucleic acid molecule according to claim 5 or 6.

20 13. A recombinant DNA construct comprising a nucleic acid molecule according to claim 5 or 6 wherein said nucleic acid molecule is inserted in the antisense orientation.

25 14. A recombinant DNA construct according to claim 12 or 13 wherein said recombinant DNA construct is an expression vector.

30 15. A construct according to claim 14 which comprises an inducible promoter.

 16. A construct according to claim 14 or 15 which comprises a sequence encoding a reporter molecule.

35 17. Cells containing a recombinant DNA construct according to any of claims 12 to 16, wherein said cells are bacterial or eukaryotic.

- 39 -

18. A nucleic acid molecule according to any of claims 1 to 8 or the nucleotide sequences illustrated in Seq ID Nos 1 to 9 for use as a medicament.

5 19. Use of a nucleic acid molecule according to any of claims 1 to 8 or the sequences illustrated in Seq ID Nos 1 to 9 in the preparation of a medicament for treating *Candida albicans* associated diseases.

10 20. A polypeptide according to claim 10 or 11 for use as a medicament.

 21. Use of a polypeptide according to claim 10 or 11 in the preparation of a medicament for treating
15 *Candida albicans* associated infections.

 22. A pharmaceutical composition comprising a nucleic acid molecule according to any of claims 1 to 8 or a polypeptide according to claim 10 or 11
20 together with a pharmaceutically acceptable carrier diluent or excipient therefor.

 23. A *Candida albicans* cell comprising an induced mutation in the DNA sequence encoding the
25 polypeptide according to claim 10.

 24. A method of identifying compounds which selectively modulate expression or functionality of polypeptides or metabolic pathways in which these
30 polypeptides are involved and which are crucial for growth and survival of *Candida albicans*, which method comprises:

 (a) contacting a compound to be tested with one or more *Candida albicans* cells having a
35 mutation in a nucleic acid molecule according to any of claims 1 to 8 which

- 40 -

mutation results in overexpression or underexpression of said polypeptides in addition to contacting one or more wild type *Candida albicans* cells with said compound,

5 (b) monitoring the growth and/or activity of said mutated cell compared to said wild type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of

10 said compound on a polypeptide or another polypeptide in the same or a parallel pathway.

25. A compound identifiable according to the

15 method of claim 24.

26. A compound according to claim 25 for use as a medicament.

20 27. Use of a compound according to claim 25 in the preparation of a medicament for treating *Candida albicans* associated diseases.

28. A pharmaceutical composition comprising a

25 compound according to claim 25 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

29. A method of identifying DNA sequences from a

30 cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or organism, which method comprises:

(a) preparing a cDNA or genomic library from said cell or organism in a suitable

35 expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription

- 41 -

of antisense RNA from the nucleotide sequences in said cDNA or genomic library.

- 5 (b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant.

10 30. A method according to claim 29 wherein said cell or organism is a yeast or filamentous fungus.

15 31. A method according to claim 29 or 30 wherein said cell or organism is any of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

32. An antibody capable of binding to a polypeptide according to claim 10 or 11.

20 33. An oligonucleotide comprising a fragment of from 10 to 120 contiguous nucleotides of a nucleic acid molecule according to any of claims 1 to 8.

25 34. An oligonucleotide according to claim 33 comprising a fragment of from 10 to 50 contiguous nucleotides.

30

35

*1/53**FIG. 1.*

AACAGCTGGT CTTCTGCTAA TACATTCAAC CCTTTCCATA TCTATACTCC
1 50

AACAATATGA TAACTGATGA ACAATTGAAT ACCATTGCAT TGACATTTGG
51 100

TTTTGCTTCA ATAATATTAA TCATAATATA TCATGCCATA TCTACTAATG
101 150

TACATAAATT AGAAGATGAA ACCCCATCAT CTTCAATTAC CAGAACAAAT
151 200

ACTACTGAAA CTAAGTTGC AAGTAAGAAA AAGAAGTAAT AACTGATGGA
201 250

TTTTTCTTCC TACCACCAAT TGAATAATGC TAGACTTGTT GGTGTGCTAC
251 300

AAATATTTCA AAAGAAAATA CGAATACTTT ATAAAATGGT AAGAACGGAA
301 350

GATGGTTTCT CATTTATACA CTAAATACAA ATCACATACA CATAACAAA
351 400

CACAAATACA TACATACACC TATATCCCTT TATTTGAT
401 438

2/53

FIG. 2.

ATGTTAAAAA CACTAACTCA AACTTTACGC TTAAGTGGGA AAGCTTTCCC
1 50

AAAGGTCCGT CCGGCCTTGA TCAGAACCTA CGCTGCCTTC GACCGTTCTA
51 100

AACCTCATGT CAACATTGGT ACTATTGGTC ATGTTGATCA TGGTAAAAC
101 150

ACATTGACTG CTGCTATCAC CAAAGTTTTA GCCGAACAAG GTGGTGCCAA
151 200

CTTCTTGAT TATGGTTCTA TTGATAGAGC TCCAGAAGAA AGAGCTAGAG
201 250

GTATCACTAT TTCCACTGCC CACGTTGAAT ACGAAACCAA GAACAGACAC
251 300

TATGCCACG TTGATTGTCC AGGACACGCT GATTATATCA AAAATATGAT
301 350

TACTGGTGCC GCTCAAATGG ATGGTGCTAT CATTGTTGTT GCTGCCACTG
351 400

ATGGTCAAAT GCCTCAAACC AGAGAACATT TGTTATTGGC CAGACAAGTT
401 450

GGTGTTC AAG ACTTGGTTGT GTTTGTCAAC AAAGTCGATA CTATTGATGA
451 500

CCCTGAAATG TTGGAATTAG TCGAAATGGA AATGAGAGAA TTGTTATCCA
501 550

CCTACGGTTT TGATGGTGAC AACACTCCAG TTATTATGGG ATCTGCTTTA
551 600

ATGGCTTTGG AAGACAAGAA ACCAGAAATT GGTAAGGAAG CTATCTTGAA
601 650

ATTGTTAGAT GCTGTCGATG AACACATTCC AACTCCATCA AGAGACTTGG
651 700

AACAACCATT TTTGTTACCA GTTGAAGACG TGTTCTCCAT CTCCGGTAGA
701 750

GGAAGTGTG TCACTGGTAG AGTTGAAAGA GGTGTTTTGA AGAAGGGTGA
751 800

AGAAATCGAA ATTGTTGGTG GTTTTGACAA ACCTTACAAG ACTACTGTGA
801 850

CCGGTATTGA AATGTTCAAA AAAGAATTAG ACTCTGCTAT GGCTGGTGAC
851 900

3/53

FIG. 2 (CONTINUED).

AACTGTGGTG TTTTGTTAAG AGGTGTTAAA AGAGATGAAA TCAAGAGAGG 950
901

TATGGTTTTG GCCAAACCAG GTACTGCTAC TTCTCACAAG AAGTTCTTGG 1000
951

CTTCCTTGTA TATTTTGA CTCCGAAGAAG GTGGTCGTTC CACTCCATTT 1050
1001

GGTGAAGGTT ACAAGCCTCA ATGCTTCTTC AGAACTAACG ATGTCACTAC 1100
1051

CACATTTTCA TTCCCAGAAG GAGAAGGTGT TGATCATTCT CAAATGATCA 1150
1101

TGCCAGGTGA CAACATTGAA ATGGTTGGTG AATTGATCAA ATCTTGTTCA 1200
1151

TTAGAAGTCA ACCAACGTTT CAACTTGAGA GAAGGTGGTA AACTGTTGG 1250
1201

TACTGGTTTG ATTACCAGAA TCATCGAATA AACAGAATGT GCACTGTGAA 1300
1251

TAATAAAAAG AAAAGAGGTA TATATAGGTG ACTTTGTATT TTGTATTGAA 1350
1301

CAATAAAATT CTGTAAATAG TAAGGGCCTC 1380
1351

4/53

FIG. 3.

GAATTCGCCCTTAAGCACTCGTTTCAACTATACATTTCAGTAACAACACCCTTAATTTACCAAACCTACA
 TTAATGGAAGTA
 ACACAACGGACGCAGAGTCAGACACAACCAACACAACAGTACCGACAACCTCAGACGCAAACCCAAAG
 CAAAGAGGACCA
 GAATAGGATTTGTCAATTGATTTGCTCCACGGGTGAGTTTGGCAATTATGATTTGAATATCAACGATA
 AAACATATCGTAC
 AAGGTAAAATGACGTGGTATTTTGGGAAGAGACCCCAACTCAGATTTGCAAGTGGCGTCGTCGTCGAGA
 ATTTCAAACAAG
 CATTTTCAAATCTGGCTCAACTTCAATGATAAATCACTATGGATAAAGGACACTTCAACTAACGGGAC
 ACACCTTAACAA
 CAGTCGATTGGTGAAAGGATCAAACCTACCTTCTTAATCAGGGTGATGAAATAGCAGTAGGGGTTGGTA
 GAGACGAGGACG
 TTGTGAGGTTTGTCTGTTGTCTTTGGTGACAAATACAACCCGGCAAAGCTACCTGATTGACCAACACA
 ATTAAAGATGAA
 GGAATATACAAAGACTTTATTGTGAAAAATGAAACGATAGGCCAAGGAGCATTGCGCACTGTGAAAA
 GGCGATTGAACG
 ATCTACGGGCGAGTCGTACGCGGTGAAGATTATAAATCGAAGAAAGCATTAAATACCGGTGGTGGAA
 GTGCCATGGCAG
 GAGTGGACCGTGAATTGTCCATATTAGAGCGGCTCAACCACCCAAATATAGTTGCTCTAAAGCTTTT
 TATGAAGATATG
 GACAATTACTATATTGTGATGGAATTGGTGCCGGGCGGTGATTTGATGGACTTTGTGGCTGCAAACGG
 TGCAATAGGAGA
 AGACGCAACACAAGTGATCAGGAAACAGATTCTAGAAGGAATTGCCTATGTTTCATAATTTAGGAATCT
 CCCATCGTGATT
 TGAAGCCAGATAATATTTTGATTATGCAAGATGACCCAATACTTGTTAAATCACCAGCTTTGGATTG
 GCAAATTCAGT
 GACAATCTGACGTTTATGAAAACCTTTTGTGGTACATTGGCGTATGTTGCTCCCGAAGTTATCACCGG
 TAAGTATGGATC
 ATCGCAGATGGAACCTGCAACAAAAGGACAACCTACTCTTCTTGGTTGACATTTGGTCTTTGGGATGTT
 TGGTTTATGTAC
 TTTTAACTTCTCATTACCATTCAACGGGAAAAACCAGCAACAAATGTTTGCCAAGATCAAAGGGGC
 GAATTTTCATGAG
 GCTCCATTAAATTCATACGACATTTCTGAAGACGGAAGAGATTTCTGCACTGCTGCCTACAGGTTAA
 TCCTAAACTAAG
 GATGACGGCTGCTGAAGCTTTGAAACATAAATGGTTGCAAGACTTGTATGAAGAGGATTCTGTCAAAT
 CATTGAGTTTAT
 CGCAATCACAGTCGCAACAATCTCGAAAGATAGATAATGGTATCCATATCGAATCATTGAGCAAAATT
 GATGAAGACGTT
 ATGCTTCGTCCATTGGATAGCGAAAGAAATAGGAAATCAAGTAAACAGCAAGATTTCAAGGTACCCAA
 GCGTGTGATTCC
 GTTATCTCAACATCCTGCAACACCGTTACCAATGTCACAACCGAAAAAGAGGCGGTATCAAATAGACC
 CTAGAACAAACA
 AAAAAGTCGATTTGGAAGAACCTCTGACAAGCAAGAAAGTCAAGCTAAGTGATTCCGTTGTTGCGGAA
 GACTACTTGAAG
 TTGGGGCCACTTGCAAATTCGTTATTCCAAGAAACAATAAATATTTCAAAGTCCCCGTTTCTTTTCGG
 AAGAAATGACAC
 TTGTGATTGCGAGATAGACGACGACAGACTATCCAACTTCATTGTGTCAATTACCAAAGAAAACGACT
 CTATATGGTTAT
 TGGATAAGAGTACTAACTCGTGCTTGGTCAACAATACTAGTGTGGAAAAGGCAACAAAGTTTGTCTT
 AGAGGAGGGGAG
 ATATTACATCTCTTCTTTGACCCATTGTCACTGCAACATATAGGTTTCAAAGTAGTCCTTGTGATCA
 ACTGTCTGGTGA
 ACATAAGAGTCAAGTGGAGGTTTGTAAACAAACCTCAGAAGAAATGAATATTATCCACTTATTTCTG
 GTTTAAGTAGTA
 TAAGTTCATAGATTTAGCATATATACAAGCATTTCTATAGAAACAAAGGTTTCAATTAATTTAGTTATT
 TACCTCCATGCA
 ATTACATTTACTTCTTCTTCCAAGGGCGAATTCTGCAGATATC

5/53

FIG. 4.

ATGGGTAGTATGTGAAGATACAATATTGAAAGTGTCTTACTAGAAATATCTAAGATGTTTGAAGCCCATGG
AC
ATTTTTGGATTGATAATTAAAAAAGTAGCAATAGATTATTGCGTTGGAGAAAGAATCACCATAGTT
GC
AAGATTTGATAGATGTTAAATGTTACGCGAGGCGAAAGATGTAACATCTCTTAAAGTAAGAAGAATA
TG
GACATGAATAAAAAATAGATAGCACTATTTTGGAACTTGTTGAAGATATTAAATAGAAATGGGATTTC
AC
ATAGATATTCAAAGTAACGAAACCTCACAAATCAAATAAAAAACAACAGTAATACTAACAATTCAATTTT
TA
TTTTTATAGAGGGTACTCCATCTTTAGGTAAACGTCACAACAAATCTCACACCTTATGTAACAGATGT
GG
CCGTCGTTTCATTCCACGTCCAAAAGAAGACCTGTTCTTCTTGTGGTTACCCAGCTGCTAAAATGAGAT
CT
CACAACCTGGGCCTTAAAAGCCAAAAGAAGAAGAACTACTGGTACCGGTAGAATGGCTTACTTGAAACA
CG
TTACCAGAAGATTCAAGAACGGTTTCCAACTGGTGTTGCTAAAGCTCAAACCCCTTCCGCTTAAACT
AA
TTACTGAAGTTATTGGTCATGCATTAGTCATTATTTCATTAAAGTCATGTTAAGCATAGCAAAGGAAGA
AT
TGGTTAGATTCTTGTTTAAATGTAATGACTATTAAATATCTGTTTAAATAAGAGGTTTAGTCTTTAT
TT
TTTTACGTATACACCAAAAAAAAAAAGAAACAAATAAAATCTGTATATTAATGTTGG

6/53

FIG. 5.

ATGGGTACTA GTACAAGTA AGCATTGAAG AACATCAAAA ACAAACAGCG
1 50

AAGACAGAAA GTTTTTCAG AAATAAAACA TGAAAAGAAT AAACAACGTC
51 100

ATAAGCAAAG AGCCGAAAGA GCTAAGGAAG AAAGAGAAAA CCCCGAATTA
101 150

AGAGAGGAAA GAATAGCAGC TAATATCCCA GATACTATAG ATAGCAAACG
151 200

TATTTATGAT GAGACTATAG CTGCTGAAGT TGAAGGAGAT GACGAGTTTC
201 250

AGTCATATTT CACTAACTTG TTGGAAGAAC CAAAGATTTT GTTGACAACA
251 300

AGTGCCAATG CTAAAAAACC GGCCTATGAA TTTGCAGACA TGATCATGGA
301 350

CTTTTTACCG AATGTGACAT TTATCAAAAG GAAGAAGGAA TATACAATGC
351 400

AAGATATGGC CAAATATTGC TCGAATAGAG ACTTCACTGC ATTGCTTGTC
401 450

ATCAACGAAG ACAAGAAGAA GGTCAATGGT ATAACGCTCA TCAATTTACC
451 500

TGAAGGGCCA ACATTTTATT TTTTCGATTAC ATCAATAGTT GATGGGAAAA
501 550

GAATTAAGGG ACACGGGAAA GCTGGTGATT ATTTACCTGA GATTGTATTG
551 600

AATAATTTCA ATTCAAGATT GGGTAAACT GTGGGAAGAC TATTTCAAAG
601 650

TATTTTCCCT CATAAACCTG AACTTCAAGG AAGACAAGTG ATTACTTTGC
651 700

ACAATCAACG TGATTATATT TTTTTCAGAA GACATAGATA TATTTTCAGA
701 750

AATGAGGAAA AGGTTGGATT GCAGGAATTG GGTCCGCAGT TTACATTAAA
751 800

GCTAAGAAGA ATGCAAAAGG GAGTACGTGG TGATGTTGTT TGGGAACACA
801 850

GACCAGATAT GGAAAGAGAT AAGAAGAAGT TTTATTTATA AGCGGGTGTA
851 900

7/53

FIG. 5 (CONTINUED).

TAAAGGTAGT AGTAGTGCCT TTATAAGTAT GTGTGTGTGT TTATGCATAG
901 950

ATGTGTAAAG AGTAATACAG CTAATTCG
951 978

8/53

FIG. 6.

AACTAATTTG TTAAACATC AATACCAAGA AGTTTTTACA ATTCAATCCC 50
1

ACATACACCA TTAATTATGA ATTCTGAAAA GATTATTGAA GTTATCATTG 100
51

CTATTTTCTT ACCACCAGTA GCTGTGTTTA TGAAATGTGG TGCCACTACC 150
101

CCATTATGGA TTAAGTTGGT ATTATGTATC TTTATTTGGT TCCCTGCTAT 200
151

CTTACATGCC TTATACGTTG TGTGAAAGA TTAAACAAAC ACCAGAGATT 250
201

TACTGCTTGA TGAATTGATT ACTCCAAAGA GTTGTGACTA GTTCCCAGTG 300
251

TGTTTTTTTT GCCTTCCAAC TTTCTTTTAC ATTTTCCAT TACTACCACT 350
301

GTCTTCCCCC CTATTTTGCA GAGTTTCAA AATTTATCCA AAACATGTGA 400
351

GTCATTAAAC CATATTATTA TAATTATTCT TTTTGTATT TTTTCCCTT 450
401

AAAACACGTT AATTTATTAA TCGTTTCGTT GTTGGTATT TTATTTTTTT 500
451

GTATTTATCA ATTGGAATAT ATATCTATAC ATGAATTTAT TATCCATTGT 550
501

ACCAATTGTT AAAACATTTT GTTAGTTTTT TGTTACTAGT ATAAAANNAT 600
551

AATAAAAGTT TANTTCAAC 619
601

9/53
FIG. 7.

ATGACATTAG GGTTCGATAA ATTCATAAGC AAGGTCAGCA CTCATAGACG
1 50
TCAATCTGAA CCATCAATCT TGGAAATCGC AGCCACCAAT TCTCAAAATA
51 100
AATCGAGAAG GCTAAGTATG GATAATGGTC ATTGTTATGT TCGTGAATCA
101 150
ACTAATAATC ATCATCATTT AAATACCGTC GTTGATAATT TACGACAGCG
151 200
TGCGGGATCG TTTTCATTTA TTTCACATCA CCATAATCAC CATCAGAATA
201 250
GTCACGATAA TTATACTGTC GATCCCCTTA CATCAAACGG AGCAGGAATT
251 300
TCCCGATCAC GTTCACGTTT CAAATCAGTT GGGCACGGAG AAGCAATATC
301 350
ACCAGCGTAT TTTTCCAAGA ATAAAACCAA AGATTTAGTG AAACAGGAAA
351 400
CAGCACATAT CATTCTGAAG AAATTACTCA ACATGTTACA AGATTTGGAT
401 450
TTACAAAACC CTATTGCATT GAAAACAATA TCACAAGGTT CAGAATCAAA
451 500
GTTTTGTAAA ATCTACGTGT CTAACACTAA TAATTGTATT TACTTACCAG
501 550
CAGCAAGTTC AACAAGTTTC ACTTATGAAG ATGATGAAAA TGGCGGCGTT
551 600
ATAATTGCTG AAGATAGAAA TGATGAAATG CCAACAGCAG TTAATAACAA
601 650
TACTTTGTCA ATGGATAGTA TAAATCATT AGAGACTGAT TTCCTGGATT
651 700
CTCCACCACC TCCAGATTTA TTTTCTAAAA TGAAATCATT CCATTCACCA
701 750
AATTACTTGA CTTCAAAAAT CGATTCTGAA TGTCCAATTC CACATACATT
751 800
TGCTGTGATT GTTGAATTAA CCAAGGACTC TTTGATTATT AAAGATCTTC
801 850
ATTTCCAATT TCAGTCATTA ACTACCATTT TATGGCCAAC TGGGGATGCA
851 900

10/53

FIG. 7 (CONTINUED 1).

TATAATCGGA CTCATGCCAA GGAGAAATTT ACCATTGGGA ATATGGAATG
901 950

GCGTACATCT TTAAGCGACG CCGACTATTA TATCAATAGT TCTAATTCCA
951 1000

ACGATGTTAA GCTGAAAAAC TTGGGTCCTG AAGATCTTAT TAATCGAACT
1001 1050

AGAGAATACA AATTAATCGA TATTGAAGAA CCAAACAATT CATCAAACAG
1051 1100

TTTACTGGAT GATGACATGG ATATTAATAA TATTACGTCG CCATTATCAA
1101 1150

CGTCACCAAC ATCAAGTTCA ACTTCAACAA ATTCAACCTC CAACTCATTG
1151 1200

GGTTCAGATT CATATAAAGC TGGTCTTTAT GTATTTTTAT TACCAATCTT
1201 1250

ATTGCCAGAA CATATTCCTG CTTCCATTGT TTCTATTAAT GGTTCATTGG
1251 1300

CTCATACATT ACTGGTTGAA TGCAATAAAT ATACTGATAA GTTGAATCGG
1301 1350

AAATCAAAAG TATCAGCATC GTACAATTTA CCTATGGTCC GTACTCCACC
1351 1400

AAACATTGGT AATTCCATTG CTGATAAGCC AATTTATGTT AATAGGATTT
1401 1450

GGAATGATGC CGTACATTAT ATTATAACTT TCCCCGCAA ATATGTTACT
1451 1500

TTGGGTTGTG AACACATGAT AAATGTGAAA TTACTGCCCA TGGTGAAAGA
1501 1550

TGTGGTTATC AAGCGTATTA AATTTAATGT ATTGGAGAGA ATAAC TTATG
1551 1600

TTTCCAAAAA TTTATCACGA GAATATGATT ATGATAGTGA AGACCCCTAT
1601 1650

TGTATTCATC CAGTTTCTAA AGAAAATAAA GTACGTGAAC GTGTTGTGTC
1651 1700

GTTATATGAA TTGAAAACGA AGGCAAAACA ATCTTCTGGT GGACATCTTG
1701 1750

AAGCTTATAA ACAAGAAGTT ATGAAATGTC CGGAAAATAA CCTTTTATTT
1751 1800

11/53

FIG. 7 (CONTINUED 2).

TCTTGTTATG AGGTTGAAAA TGATAATAAT AACGGCAACG GCAACGGCAA
1801 1850

CGGCAACGGA AACAAGAACG TTAAACAAAA GAATAAAGAT CAACCAATGA
1851 1900

TTGCTACACC TTTAGATATC AATGTTTCTT TACCATTTTT AACTACTATG
1901 1950

TCTGATAGTT TAATTATGAC ATCAGCCATA GAAGAAGAAG GTTCAGATCT
1951 2000

GCCTCATACA TCAAGAAGAG GGTCGGCAGT GAGTATGACT GATAATAATA
2001 2050

CTACCCCAAG TAACAATAAC CCTTTATCTC CATTTTTGGG AGCAGTGGAA
2051 2100

ACTAATGGTG CTAGTATAAA TGAAATTGGT GATCATACAT TATTCCTGA
2101 2150

TTCTAATTTT CGACATATTG AAATTAAACA TCGATTACAA GTTACATTTA
2151 2200

GGATTTCTAA ACCGGATCTG GATAATAAAA TGCATCATTG TGAAGTGGTT
2201 2250

ATTGATACCC CCATCGTTTT ACTTAGTTCA AAATGTCAAG AAGATTCTCC
2251 2300

TCCTCCTTAT AGTTCTGTA
2301 2319

*12/53**FIG. 8.*

AACGTTTCGTG CAAAAGGCTA TACTGGTGAT ATCCACGCAG ATGAAGAGCA
1 50

AGTTTAATCA ACTCTTTGTC AATTAATGCT GTACTTGTTT TCATTTTATT
51 100

TGCTGGCATT TAAAGAATAC CCATAGTTCA GAAAATAAAA TTGAAAAATT
101 150

TAAAAAAAAA CGCAATATCA TTCATTTTTT TTGTTTTTTT GACAATAATA
151 200

TTAATATGTA GTTACCAATG TTTTATGATT TTATATGTTT TGAAAAAATA
201 250

GTTTG
251

FIG. 9.

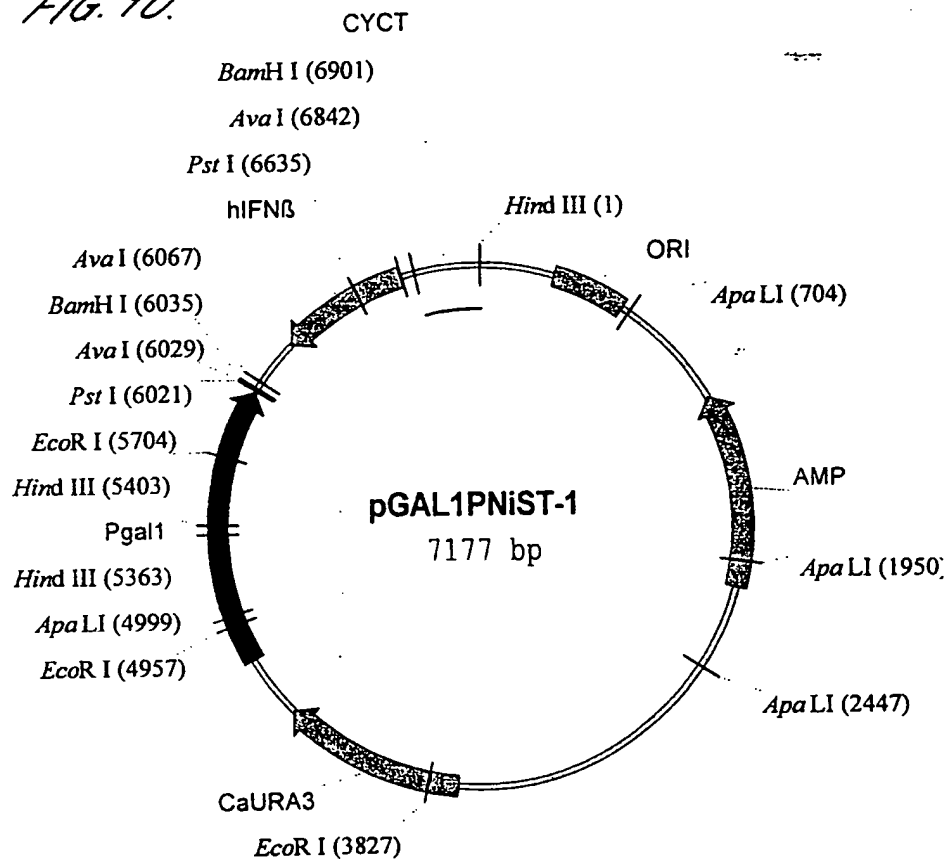
AACCTTACAA TCATTATACC AACTATCAAA ATCATAAGAC TCTTNAACTT
1 50

CTGTTTTTGA TAGTTGGTAT AATGATTTAT GTATTATCTT AATTCATTAT
51 100

TATTAGTTTC GGTCACAAA
101 119

13/53

FIG. 10.



14/53
FIG. 11.

1 AGCTTGAGTA TTCTATAGTG TCACCTAAAT AGCTTGGCGT AATCATGGTC
51 ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA
101 TACGAGCCGG AAGCATAAAG TGTAAGCCT GGGGTGCCTA ATGAGTGAGC
151 TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA
201 CCTGTCTGTC CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG
251 GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC
301 TCGGTCGTTT GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT
351 ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA
401 AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT
451 TTCCATAGGC TCCGCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG
501 TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC
551 CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA
601 TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC
651 ACGCTGTAGG TATCTCAGTT CCGTGTAGGT CGTTCGCTCC AAGCTGGGCT
701 GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC
751 TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC
801 AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG
851 AGTTCTTGAA GTGGTGGCCT AACTACGGCT AACTAGAAG GACAGTATTT
901 GGTATCTGCG GTCTGCTGAA GCCAGTTACC TTCGAAAAA GAGTTGGTAG
951 CTCTTGATCC GGCAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT
1001 GCAAGCAGCA GATTACGCGC AGAAAAAAG GATCTCAAGA AGATCCTTTG
1051 ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT CACGTTAAGG
1101 GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTACCTAG ATCCTTTTAA
1151 ATTAAAAATG AAGTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG
1201 TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG
1251 TCTATTTCTG TCATCCATAG TTGCCTGACT CCCCCTCGTG TAGATAACTA
1301 CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA

15/53

FIG. 11 (CONTINUED 1).

1351 GACCCACGCT CACCGGCTCC AGATTATCA GCAATAAACC AGCCAGCCGG
1401 AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT
1451 CTATTAAATG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT
1501 TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC
1551 GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA
1601 CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG
1651 ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC
1701 AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG
1751 TGA CTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA
1801 CCGAGTTGCT CTTGCCCCGGC GTCAATACGG GATAATACCG CGCCACATAG
1851 CAGAACTTTA AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC
1901 TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT
1951 GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG
2001 AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC
2051 GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT
2101 TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA
2151 AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCTG
2201 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT
2251 ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG GTGAAAACCT
2301 CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG TAAGCGGATG
2351 CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT
2401 CGGGGCTGGC TTA ACTATGC GGCATCAGAG CAGATTGTAC TGAGAGTGCA
2451 CCATATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA AAATACCGCA
2501 TCAGGCGAAA TTGTAAACGT TAATATTTTG TTA AAAATTCG CGTTAAATAT
2551 TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC
2601 CTTATAAATC AAAAGAATAG ACCGAGATAG GGTGAGTGT TGTTCAGTT
2651 TGGAACAAGA GTCCACTATT AAAGAACGTG GACTCCAACG TCAAAGGGCG

16/53

FIG. 11 (CONTINUED 2).

2701 AAAAACCGTC TATCAGGGCG ATGGCCCACT ACGTGAACCA TCACCCAAAT
2751 CAAGTTTTTT GCGGTCGAGG TGCCGTAAAG CTCTAAATCG GAACCCTAAA
2801 GGGAGCCCCC GATTAGAGC TTGACGGGGA AAGCCGGCGA ACGTGGCGAG
2851 AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG CTGGCAAGTG
2901 TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG
2951 CTACAGGGCG CGTCCATTCG CCATTCAGGC TGCGCAACTG TTGGGAAGGG
3001 CGATCGGTGC GGGCCTCTTC GCTATTACGC CAGCTGGCGA AAGGGGGATG
3051 TGCTGCAAGG CGATTAAAGT GGGTAACGCC AGGGTTTTCC CAGTCACGAC
3101 GTTGTA AAC GACGGCCAGT GAATTGTAAT ACGACTCACT ATAGGGCGAA
3151 TTGGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG TGGCGCGGTA
3201 TTATCCCGTG TTGACGCCCG GCAAGAGCAA CTCGGTCGCC GCATACACTA
3251 TTCTCAGAAT GACTTGGTTG AGTACTAATA GGAATTGATT TGGATGGTAT
3301 AAACGGAAAC AAAAAAAGA GCTGGTACTA CTTTCTTTAA AATTATTTTA
3351 TTATTTGATT TTATTTAATA GTATATATTA TATTTTGAAC GTAGATTATT
3401 TTGTTGAAAG TTGCTGTAGT GCCATTGATT CGTAACACTA ATTCTGTATT
3451 AGTCATTCCT CTTGTTTGAT AGTATCCAAA AAAACGGCTA TTTTTTTGCA
3501 ATCTTATTTT CTGCATATTA TACAGATAAC ATAATGAAAG AAAAAATCTT
3551 TTTTTTTGTT CTTCAATGAT GATTTCACC ATTCTTTTAA ACATTGATCA
3601 ATTCCTGAGC AACAACCCCA TACACACTGG TTTATATACC GCCCCTTTTA
3651 CAGTTGAAGA AAGAAATAGA AATAGAAATA GCAAACAAAA GATATGACAG
3701 TCAACACTAA GACCTATAGT GAGAGAGCAG AAATCATGC CTCACCAGTA
3751 GCACAGCGAT TATTTGATT AATGGAAGT AAGAAAACCA ATTTATGTGC
3801 ATCAATTGAC GTTGATACCA CTAAGGAATT CCTTGAATTA ATTGATAAAT
3851 TAGGTCCTTA TGTATGCTTA ATCAAGACTC ATATTGATAT AATCAATGAT
3901 TTTTCCTATG AATCCACTAT TGAACCATTA TTAGAACTTT CACGTAAACA
3951 TCAATTTATG ATTTTGAAG ATAGAAAATT TGCTGATATT GGTAATACCG
4001 TAAAGAAACA ATATATTGGT GGAGTTTATA AAATTAGTAG TTGGGCAGAT

17/53

FIG. 11 (CONTINUED 3).

4051 ATTACCAATG CTCATGGTGT CACTGGGAAT GGAGTGGTTG AAGGATTAAA
4101 ACAGGGAGCT AAAGAAACCA CCACCAACCA AGAGCCAAGA GGGTTATTGA
4151 TGTTAGCTGA ATTATCATCA GTGGGATCAT TAGCATATGG AGAATATTCT
4201 CAAAAAACTG TTGAAATTGC TAAATCCGAT AAGGAATTTG TTATTGGATT
4251 TATTGCCCAA CGTGATATGG GTGGCCAAGA AGAAGGATTT GATTGGCTTA
4301 TTATGACACC TGGAGTTGGA TTAGATGATA AAGGTGATGG ATTAGGACAA
4351 CAATATAGAA CTGTTGATGA AGTTGTTAGC ACTGGAAC TG ATATTATCAT
4401 TGTTGGTAGA GGATTGTTTG GTAAAGGAAG AGATCCAGAT ATTGAAGTA
4451 AAAGGTATAG AAATGCTGGT TGAATGCTT ATTTGAAAAA GACTGGCCAA
4501 TTATAAATGT GAAGGGGGAG ATTTTCACTT TATTAGATTT GTATATATGT
4551 AGAATAAATA AATAAATAAG TTAAATAAAT AATTAAATAA GGGTGGTAAT
4601 TATTACTATT TACAATCAAA GGTGGTCCTT CTAGCTGTAA TCCGGGCAGC
4651 GCAACGGAAC ATTCATCAGT GTAAAAATGG AATCAATAAA GCCCTGCGCA
4701 GCGCGCAGGG TCAGCCTGAA TACGCGTTTA ATGACCAGCA CAGTCGTGAT
4751 GGCAAGGTCA GAATAGCCCA AGTCGGCCGA GGGGCCTGTA CAGTGAGGGA
4801 AGATCTGATA TTGACGAAGA GGAACCAATG TAACGTTACA CTGAAGAAAA
4851 CACATAATAA ACGGGAAGAA ACGGTGTAAA AGTGTGAAAA TAATTTTGA
4901 ATATCATTTT CCTTGGTTTA ATTCCAAACG AAACGTGTAT TTTTITAGAG
4951 AATGGGAATT CTTATTGGAT GTCTAGATTG TTTGTTTACT CCAGACTGTG
5001 CACAAAAACG TTTGGATGGA TGATCAGAAG ATATTTTTTAG GCTTAGCTCT
5051 AAATATAAGA AATGATGCTT GAAAATCCAG ACAGAAATTG AGTTTCAAAA
5101 ATTGGTAATG TGAGGTATTA GTCAACTAAC CAAATAACAA TGCAAACCGG
5151 TTGATACATT TCATTTTGAA AATAATGAAA CTGGAATTGG ATGACCAGCA
5201 CACAAACACA TAAAGTAATT ATGGGAATTA GAAGCGAACA TAGAGGAATA
5251 CTTTGCCACG AACAGAATAC AAGTGGAAC ACTTTTTTCT CCATTGTTTT
5301 AGTTCTGTTT TTTTGTCAA CTGGTTTTGT GCTATGTGTA AAAAAATATT
5351 GCCAAGAAAA AAAGCTTGTT TTGTGGCCAG TGTCGAAAAA AAATTTTGGG

18/53

FIG. 4 (CONTINUED 4).

5401 GAAGCTTCGG ATTAATTTAT TTTTATTTC CATCGGGGAA AGTGGGGGGG
5451 AAAAAAATT TAAGCAGTTC ATAAACCTT CAAAAAATA TATGGACAGA
5501 GATGATTGTA TTTTCCCGAC ACCAAAATCA TAATTAATA TGAGAAAATT
5551 GAATGTAACG TTACAATTTA TTTTATTTG AAGCTGAAAA GCGATTTATG
5601 ATTTTCCGA AATGAAAATT TTTTATAGGT TTATTTTTT TGTCGGGCAA
5651 AGAAAACTG AACAAGGATT ATTAAATTT TTGGTGTGTTG TTTGTGTCTG
5701 GAGAATTCAT TCCTCTCTCA TCTTCACACA ATGTTTAGAC ATCTGACACG
5751 ATTCAAAATA GTTCGGTTTC CGGGGTTGGT GTTTAGTTTT CGTTTTTCGT
5801 TTTTGTGGA AAGAATGTTT TAGCTCATG GTTTCTTTC TTCATTCAAT
5851 AGTTTGAAA GAATTTGCCC ACTTGTTATT ACAATCATAT AAAATTAAAC
5901 TTTGATATAA AATAGAGTTT GAAAGTTTCC CAGATCCTTT TGATTTCTT
5951 TGTAATTTTT TTTTCTCCA CATATACACA CATAAAACC GATTTTTATA
6001 AGAAAGAGTT ATACCCTGCA GCTCGACCTC GAGGGATCCG GGCCCTCTAG
6051 ATGCGGCCGC TAGGCCTCGA GGGACTTTTG CACCAAAAAT AATTTATTTT
6101 CCAAAATAAA ATTTAAATAA ATAAAAATAA CTCATAATTT AATAAAAATT
6151 TCAAAATCTT CTAGTGTCTT TTCATATGCA GTACATTAGC CATCAGTCAC
6201 TTAAACAGCA TCTGCTGGTT GAAGAATGCT TGAAGCAATT GTCCAGTCCC
6251 AGAGGCACAG GCTAGGAGAT CTTAGTTTC GGAGGTAACC TGTAAGTCTG
6301 TTAATGAAGT AAAAGTTTCT TAGGATTTCC ACTCTGACTA TGGTCCAGGC
6351 ACAGTGACTG TACTCCTTGG CCTTCAGGTA ATGCAGAATC CTCCCATAAT
6401 ATCTTTTCAG GTGCAGACTG CTCATGAGTT TTCCCTGGT GAAATCTTCT
6451 TTCTCCAGTT TTTCTTCCAG GACTGTCTTC AGATGGTTTA TCTGATGATA
6501 GACATTAGCC AGGAGGTTCT CAACAATAGT CTCATTCCAG CCAGTGCTAG
6551 ATGAATCTTG TCTGAAAATA GCAAAGATGT TCTGGAGCAT CTCATAGATG
6601 GTCAATGCGG CGTCCTCCTT CTGGAAGTGC TGCAGCTGCT TAATCTCTC
6651 AGGGATGTCA AAGTTCATCC TGTCCTGAG GCAGTATTCA AGCCTCCCAT
6701 TCAATTGCCA CAGGAGCTTC TGACACTGAA AATTGCTGCT TCTTTGTAGG

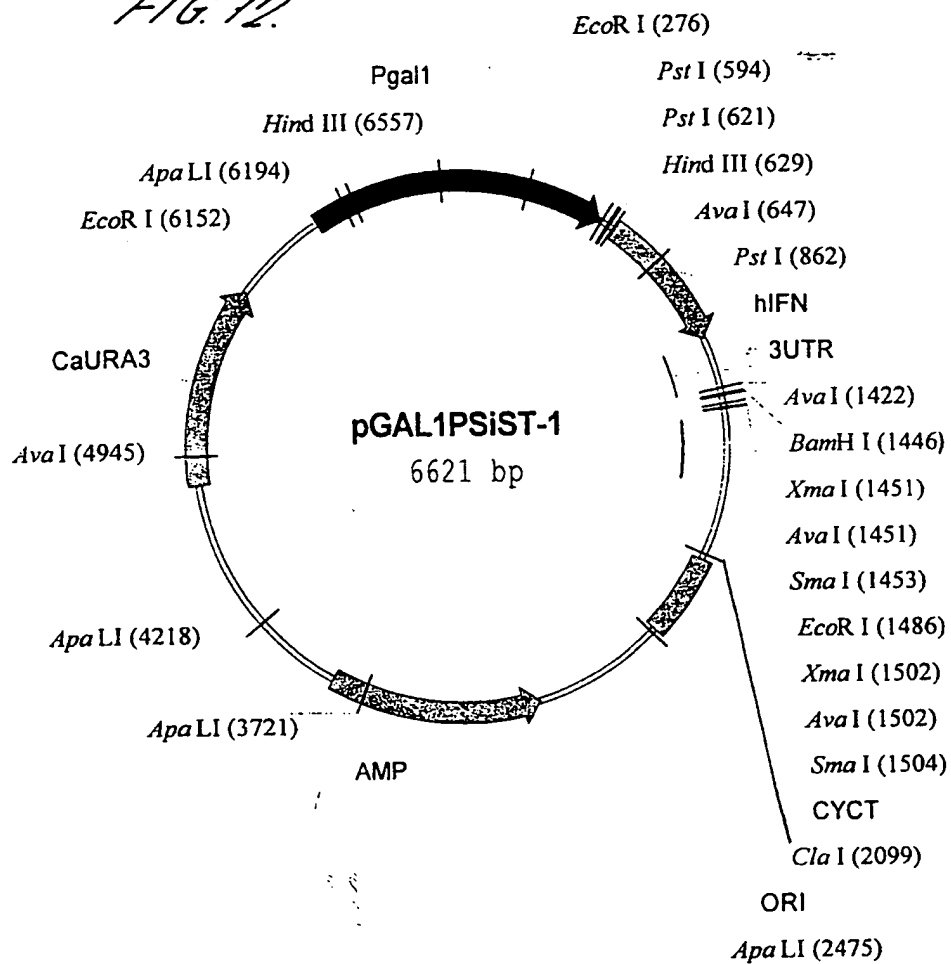
19/53

FIG. 11 (CONTINUED 5).

6751 AATCCAAGCA AGTTGTAGCT CATGGAAAGA GCTGTAGTGG AGAAGCACAA
6801 CAGGAGAGCA ATTTGGAGGA GACACTTGTT GGTGATGTTT CTCGAGGCCT
6851 TTTTGGCCAG CTGGCGCCTG CTGCGCGACG GCGAGCTGCT CACCACCCAG
6901 GATCCGTCCC CCTTTTCCTT TGTCGATATC ATGTAATTAG TTATGTCACG
6951 CTTACATTCA CGCCCTCCCC CCACATCCGC TCTAACCGAA AAGGAAGGAG
7001 TTAGACAACC TGAAGTCTAG GTCCCTATTT ATTTTTTTAT AGTTATGTTA
7051 GTATTAAGAA CGTTATTTAT ATTTCAAATT TTTCTTTTTT TTCTGTACAG
7101 ACGCGTGTAC GCATGTAACA TTATACTGAA AACCTTGCTT GAGAAGGTTT
7151 TGGGACGCTC GAAGGCTTTA ATTTGCA

20/53

FIG. 12.



21/53

FIG. 13.

1 TTCCATCGGG GAAAGTGGGG GGGAAAAAAT TTTAAGCAGT TCACAAAACC
51 TTCCAAAAAA TATATGGACA AAGATGATTG TATTTTCCCG ACACCAAAAT
101 CATAATTAAT TATGAGAAAG TTAAATGTAA CGTTACAATT TATGTTTATT
151 TGAAGGTGAA AAGCGATTTA TGATTTTCC GAAATGAAAA TTTTTTTTAG
201 GTTTATTTTT TTTGTCGGGC AAAGAAAAAC TGAACAAGGA TTATTAAAAT
251 TTTTGGTGTT TGTTTGTC TGGAGAATTC ATTCCTCTCT CATCTTCACA
301 CAATGTTTAG ACATCTGACA CGATTCATGA TAGTTCGGTT TCCGGGGTTG
351 GTGTTTAGTT TTCGTTTTTC TTTTTTTTGG GAAAGAATGT TTTAGCTCAT
401 TGGTTTTCTT TCTTCATTCA ATAGTTTTGA AAGAATTGTC CCACTTGTTA
451 TTACAATCAT ATAAAATTAA ACTTTGATAT AAAATAGAGT TTGAAAGTTT
501 CCCAGATCCT TTTTGATTTC TTTGTAAATT TTTTTTCTC CCACATATAC
551 ACACATACAA ACCGATTTTT ATAAGAAAGA GTTATACCCT GCAGCTCGAC
601 CTCGACTGTT TAAACCTGCA GGCATGCAAG CTTGGCCAAA AAGGCCTCGA
651 GGAACATGAC CAACAAGTGT CTCCTCCAAA TTGCTCTCCT GTTGTGCTTC
701 TCCACTACAG CTCTTTCCAT GAGCTACAAC TTGCTTGGAT TCCTACAAAG
751 AAGCAGCAAT TTTCAGTGTC AGAAGCTCCT GTGGCAATTG AATGGGAGGC
801 TTGAATACTG CCTCAAGGAC AGGATGAACT TTGACATCCC TGAGGAGATT
851 AAGCAGCTGC AGCAGTTCCA GAAGGAGGAC GCCGCATTGA CCATCTATGA
901 GATGCTCCAG AACATCTTTG CTATTTTCAG ACAAGATTCA TCTAGCACTG
951 GCTGGAATGA GACTATTGTT GAGAACCTCC TGGCTAATGT CTATCATCAG
1001 ATAAACCATC TGAAGACAGT CCTGGAAGAA AACTGGAGA AAGAAGATTT
1051 CACCAGGGGA AACTCATGA GCAGTCTGCA CCTGAAAAGA TATTATGGGA
1101 GGATTCTGCA TTACCTGAAG GCCAAGGAGT ACAGTCACTG TGCCTGGACC
1151 ATAGTCAGAG TGGAAATCCT AAGGAACTTT TACTTCATTA ACAGACTTAC
1201 AGGTTACCTC CGAAACTGAA GATCTCCTAG CCTGTGCCTC TGGGACTGGA
1251 CAATTGCTTC AAGCATTCTT CAACCAGCAG ATGCTGTTTA AGTGACTGAT
1301 GGCTAATGTA CTGCATATGA AAGGACACTA GAAGATTTTG AAATTTTTAT

22/53

FIG. 13 (CONTINUED 1).

1351 TAAATTATGA GTTATTTTAA TTTATTTAAA TTTTATTTTG GAAAATAAAT
1401 TATTTTTTGGT GCAAAAGTCC CTCGAGGCCT AGCGGCCGCC TAGAGGATCC
1451 CCGGGCGCTA GGCGGCCGCT AGGCCTTTTT GGCCGAATTC GAGCTCGGTA
1501 CCCGGGGAGA TCCGTCCCCC TTTTCCTTTG TCGATATCAT GTAATTAGTT
1551 ATGTCACGCT TACATTCACG CCCTCCCCC ACATCCGCTC TAACCGAAAA
1601 GGAAGGAGTT AGACAACCTG AAGTCTAGGT CCCTATTTAT TTTTTTATAG
1651 TTATGTTAGT ATTAAGAACG TTATTTATAT TTCAAATTT TCTTTTTTTT
1701 CTGTACAGAC GCGTGACGC ATGTAACATT ATACTGAAAA CCTTGCTTGA
1751 GAAGGTTTTG GGACGCTCGA AGGCTTTAAT TTGCAAGCTA GCTTGGCGTA
1801 ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG CTCACAATTC
1851 CACACAACAT ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA
1901 TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA
1951 GTCGGGAAAC CTGTGCTGCC AGAGATCTCT GCATTAATGA ATCGGCCAAC
2001 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC
2051 ACTGACTCGC TGCCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGATCG
2101 ATCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG
2151 CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA
2201 AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA
2251 TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT
2301 AAAGATACCA GCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT
2351 CCGACCTGCG CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTGCGGAAG
2401 CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
2451 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCCGAC
2501 CGCTGCGCCT TATCCGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
2551 CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
2601 GGTATGTAGG CCGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
2651 TACTAGTAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC

23/53

FIG. 13 (CONTINUED 2).

2701 CTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
2751 GTAGCGGTGG TTTTTTGTG TGCAAGCAGC AGATTACGCG CAGAAAAAAA
2801 GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG
2851 GAACGAAAAC TCACGTAAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA
2901 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
2951 AGTATATATG AGTAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA
3001 GGCACCTATC TCAGCGATCT GTCTATTTCTG TTCATCCATA GTTGCTGAC
3051 TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC
3101 AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC
3151 AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTGCAA
3201 CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGA AGCTAGAGTA
3251 AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTGCCA TTGCTACAGG
3301 CATCGTGGTG TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT
3351 CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAGCG
3401 GTTAGCTCCT TGGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGAGT
3451 GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC
3501 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC
3551 TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCCG CGTCAATACG
3601 GGATAATACC GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA
3651 AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC
3701 AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC
3751 TTTCACCAGC GTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA
3801 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCTT
3851 TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA
3901 CATATTTGAA TGTATTTAGA AAAATAACA AATAGGGGTT CCGCGCACAT
3951 TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA
4001 TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC TCGCGCGTTT

24/53

FIG. 13 (CONTINUED 3).

4051 CCGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
4101 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG
4151 TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA
4201 GCAGATTGTA CTGAGAGTGC ACCATATCGA CGCTCTCCCT TATGCGACTC
4251 CTGCATTAGG AAGCAGCCCA GTAGTAGGTT GAGGCCGTTG AGCACCGCCG
4301 CCGCAAGGAA TGGTGCATGC AAGGAGATGG CGCCCAACAG TCCCCCGGCC
4351 ACGGGGCCTG CCACCATACC CACGCCGAAA CAAGCACTAA TAGGAATTGA
4401 TTTGGATGGT ATAAACGGAA ACAAAAAAAA GAGCTGGTAC TACTTTCTTT
4451 AAAATTATTT TATTATTGA TTTTATTAA TAGTATATAT TATATTTTGA
4501 ACGTAGATTA TTTTGTGAA AGTTGCTGTA GTGCCATTGA TTCGTAACAC
4551 TAATTCTGTA TTAGTCATTC CTCTGTTTG ATAGTATCCA AAAAAACGGC
4601 TATTTTTTTG CAATCTTATT TCCTGCATAT TATACAGATA ACATAATGAA
4651 AGAAAAATC TTTTTTTTG TTCTTCAATG ATGATTTCOA CCATTCTTTT
4701 AAACATTGAT CAATTCCTGA GCAACAACCC CATAACACT GGTTTATATA
4751 CCGCCCCCTT TACAGTTGAA GAAAGAAATA GAAATAGAAA TAGCAAACAA
4801 AAGATATGAC AGTCAACACT AAGACCTATA GTGAGAGAGC AGAACTCAT
4851 GCCTCACCAG TAGCACAGCG ATTATTTTGA TTAATGGAAC TGAAGAAAA
4901 CAATTTATGT GCATCAATTG ACGTTGATAC CACTAAGGAG TTCCTCGAGT
4951 TAATTGATAA ATTAGGTCCT TATGTATGCT TAATCAAGAC TCATATTGAT
5001 ATAATCAATG ATTTTCCTA TGAATCCACT ATTGAACCAT TATTAGAACT
5051 TTCACGTAAA CATCAATTGA TGATTTTGA AGATAGAAAA TTGCTGATA
5101 TTGGTAATAC CGTAAAGAAA CAATATATTG GTGGAGTTTA TAAAATTAGT
5151 AGTTGGGCAG ATATTACCAA TGCTCATGGT GTCAGTGGGA ATGGAGTGGT
5201 TGAAGGATTA AAACAGGGAG CTAAAGAAAC CACCACCAAC CAAGAGCCAA
5251 GAGGGTTATT GATGTTAGCT GAATTATCAT CAGTGGGATC ATTAGCATAT
5301 GGAGAATATT CTCAAAAAAC TGTTGAAATT GCTAAATCCG ATAAGGAATT
5351 TGTTATTGGA TTTATTGCCC AACGTGATAT GGGTGGCCAA GAAGAAGGAT

25/53

FIG. 13 (CONTINUED 4).

5401 TTGATTGGCT TATTATGACA CCTGGAGTTG GATTAGATGA TAAAGGTGAT
5451 GGATTAGGAC AACAAATATAG AACTGTTGAT GAAGTTGTGA GCACTGGAAC
5501 TGATATTATC ATTGTTGGTA GAGGATTGTT TGGTAAAGGA AGAGATCCAG
5551 ATATTGAAGG TAAAAGGTAT AGAAATGCTG GTTGAATGC TTATTTGAAA
5601 AAGACTGGCC AATTATAAAT GTGAAGGGGG AGATTTTCAC TTTATTAGAT
5651 TTGTATATAT GTAGAATAAA TAAATAAATA AGTTAAATAA ATAATTAAAT
5701 AAGGGTGGTA ATTATTACTA TTTACAATCA AAGGTGGTCC TTCTAGCTGT
5751 AATCCGGGCA GCGCAACGGA ACATTCATCA GTGTAAAAAT GGAATCAATA
5801 AAGCCCTGCG CTCATGAGCC CGAAGTGGCG AGCCCGATCT TCCCCATCGG
5851 TGATGTGCGC GATATAGGCG CCAGCAACCG CACCTGTGGC GCCCGAGCGC
5901 GCAGGGTCAG CCTGAATACG CGTTTAAATGA CCAGCACAGT CGTGATGGCA
5951 AGGTCAGAAT AGCCCAAGTC GGCCGAGGGG CCTGTACAGT GAGGGAAGAT
6001 CTGATATTGA CGAAGAGGAA CCAATGTAAC GTTACACTGA AGAAAACACA
6051 CAATAAACGG GAAGAAACGG TGAAAAGTG TGAAAATAAT TTTTGAATAT
6101 CATTTCCCTT GGTTTAATTC CAAACGAAAC GTGTTTTTTT TAGAGAATGG
6151 GAATTCTTAT TGGATGTCTA GATTGTTTGT TTA CTCCAGA CTGTGCACAA
6201 AAACGTTTGG ATGGATGATC AGAAGATATT TTAGGCTTA GCTCTAAATA
6251 TAAGAAATGA TGCTTGAAAA ACCAGACAGA AATTGAGTTT CAAAAATTGG
6301 TAATGTGAGG TATTAGTCAA CTAACCAAAT AACAATGCAA ACCGGTTGAT
6351 ACATTTTATT TTGAAAATAA TGAACTGGA ATTGGATGAC CAGCACACAA
6401 ACACATAAAG TAATTATGGG AATTAGAAGC GAACATAGAG GAGTACTTGG
6451 CCACGAACAG AATACAAGTG GGAACACTAT TTTCTCCATT GTTTTAGTTC
6501 TGTTTTTTTG TCAGCCTAGT TTTGTGCTAT GTGTAAAAAA TATTGCCAAG
6551 AAAAAAAGCT TGTTTTGTGG CCAGTGTCCG AAAAAAATTT TGGGGAATCT
6601 TCGGATTAAT TTATGTTTTTC A

*26/53**FIG. 14.*

MITDEQLNTI ALTFGFASII LIIYHAIST NVHKLEDETP SSSFTRTNTT
1 50

ETTVASKKKK
51 60

FIG. 15.

MLKTLTQTLR LTGKAFPKVR PALIRTYAAF DRSKPHVNIG TIGHVDHGKT
1 50

TLTAAITKVL AEQGGANFLD YGSIDRAPEE RARGITISTA HVEYETKNRH
51 100

YAHVDCPGHA DYIKNMITGA AQMDGAIIVV AATDGQMPQT REHLLLARQV
101 150

GVQDLVVFN KVDTIDDPPEM LELVEMEMRE LLSTYGFDGD NTPVIMGSAL
151 200

MALEDKKPEI GKEAILKLLD AVDEHIPTPS RDLEQPFLLP VEDVFSISGR
201 250

GTVVTGRVER GVLKKGEEIE IVGGFDKPYK TTVTGIEMFK KELDSAMAGD
251 300

NCGVLLRGVK RDEIKRGMVL AKPGTATSHK KFLASLYILT SEEGGRSTPF
301 350

GEGYKPQCFE RTNDVTTTFS FPEGEGVDHS QMIMPGDNIE MVGELIKSCP
351 400

LEVNQRFNLR EGGKTVGTGL ITRIIE
401 426

27/53

FIG. 16.

MEVTQRTQSQ TQPTQQSPTT QTQTQSKEDQ NRICQLICST GQFGNYDLNI
 1 50
 NDKTIVQGKM TWYFGRDPNS DLQVASSSRI SNKHFQIWLN FNDKSLWIKD
 51 100
 TSTNGTHLNN SRLVKGSNYL LNQGDEIAVG VGRDEDVVRF VVVFQDKYNP
 101 150
 AKLPDSTNTI KDEGIYKDFI VKNETIGQGA FATVKKAIER STGESYAVKI
 151 200
 INRRKALNTG GGSAMAGVDR ELSILERLNH PNIVALKAFY EDMDNYYIVM
 201 250
 ELVPGGDLMD FVAANGAIGE DATQVITKQI LEGIAYVHNL GISHRDLKPD
 251 300
 NILIMQDDPI LVKITDFGLA KFSDNSTFMK TFCGTLAYVA PEVITGKYGS
 301 350
 SQMESQQKDN YSSLVDIWSL GCLVYVLLTS HLPFNGKNQQ QMFAKIKRGE
 351 400
 FHEAPLNSYD ISEDGRDFLQ CCLQVNPCLR MTAAEALKHK WLQDLYEEDS
 401 450
 VKSLSLSQSQ SQQSRKIDNG IHIESLSKID EDVMLRPLDS ERNRKSSKQQ
 451 500
 DFKVPRVIP LSQHPATPLP MSQPKRPPYQ IDPRTNKKVD LEEPSTSKKV
 501 550
 KLSDSVVAED YLKLEPLANS LFQETINISK SPFSFGRNDT CDCEIDDDRL
 551 600
 SKLHCVITKE NDSIWLLDKS TNSCLVNNTS VGKGNKVLLR GGEILHLFFD
 601 650
 PLSSQHIGFK VVLVDQSSGE HKSQVEVLKQ TSEEMNIPL ISGLSSISS
 651 699

*28/53**FIG. 17.*

MGTSTSEALK NIKNKQRRQK VFAEIKHEKN KQRHKQRAER AKEERENPEL
1 50

REERIAANIP DTIDSKRIYD ETIAAEVEGD DEFQSYFTNL LEEPKILLTT
51 100

SANAKKPAYE FADMIMDFLP NVTFIKRKKE YTMQDMAKYC SNRDFTALLV
101 150

INEDKKKVNG ITLINLPEGP TFYFSITSIV DGKRIKGHGK AGDYLPEIVL
151 200

NNFNSRLGKT VGRLFQSIFP HKPELQGRQV ITLHNQRDYI FFRRHRYIFR
201 250

NEEKVGLQE GPQFTLKLRRM QKGVRGDVVW EHRPDMERDK KKFYL
251 295

FIG. 18.

MNSEKIIIEVI IAIFLPPVAV FMKCGATTPL WINLVLCIFI WFPAILHALY
1 50

VVLKD
51

29/53
FIG. 19.

MTLGFDKFIS KVSTHRRQSE PSILEIAATN SQNKSRRLSM DNGHCYVRES

1

50

TNNHHHLNTV VDNLRQRAGS FSFISHHHNH HQNSHDNYTV DPLTSNGARI

51

100

SRSRSRKSV GHGEAISPAY FSKNKTDLV KQETAHIISK KLLNMLQDL

101

150

LQNPIALKTI SQGSESKFCK IYVSNTNCCI YLPAASSTSF TYEDDENG

151

200

IIAEDRNDEM PTAVNNNTLS MDSINHSETD FSDSPPPDL FSKMKS

201

250

NYLTSKIDSE CPIPHTFAVI VELTKDSLII KDLHFQFQSL TTILWPTG

251

300

YNRTHAKEKF TIGNMEWRTS LSDADYYINS SNSNDVKSXN LGPEDLIN

301

350

REYKLIDIEE PNSSNSLSD DDMDINNITS PLSTSPTSSS TSTNSTNSL

351

400

GSDSYKAGLY VFLLPILLPE HIPASIVSIN GSLAHTLSVE CNKYTDKLN

401

450

KSKVSASYNL PMVRTPPNIG NSIADKPIYV NRIWNDVHY IITFPRKYV

451

500

LGCEHMINVK LSPMVKDVI KRIKFNVLER ITYVSKNLSR EYDYDSEDP

501

550

CIHPVSKENK VRERVVSLYE LKTKAQSSG GHLEAYKQEV MKCPENLLF

551

600

SCYEVENNNG NGNGNGNG NKNVKQKNKD QPMIATPLDI NVSLPFLTT

601

650

SDSLIMTSAI EEEGSDSPHT SRRGSAVSMT DNNTTPSNNN PLSPFLGAVE

30/53

FIG. 19 (CONTINUED).

651

700

TNGASINEIG DHTLFPDSNF RHIEIKHRLQ VTFRISKPDS DNKMHYEVV
701

750

IDTPIVLLSS KCQEDSPPPY SSV
751

773

FIG. 20.

MGEGETPSLGKRHNKSHTLNRCGRRSFHVQKKTSSCGYPAAKMRSHNWALKAKRRRTTGTGRMAYLK
HV
TRRFKNGFQTGVAKAQTPSA

31/53

FIG. 21.

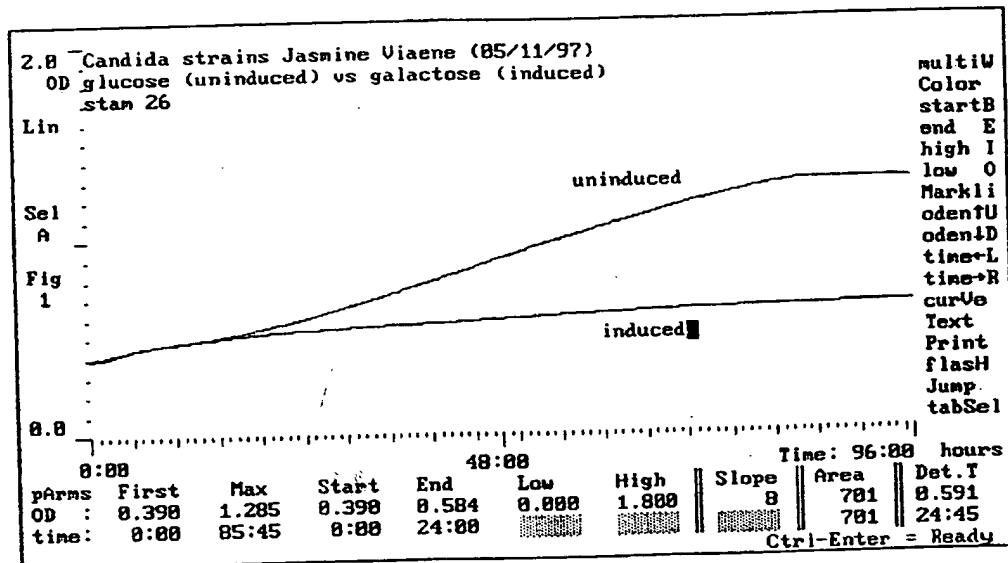
Project : Identification of novel essential genes in *C. albicans*

Strain no. : 26g

Freezer location : glycerol stocks box XXIII; C8

Growth curve(s) (Bioscreen) :

Date : 05/11/1997



Plasmid/clone name* : 26g3

Freezer location : original stocks box

Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the *Candida albicans* Access dbase

32/53

FIG. 22.

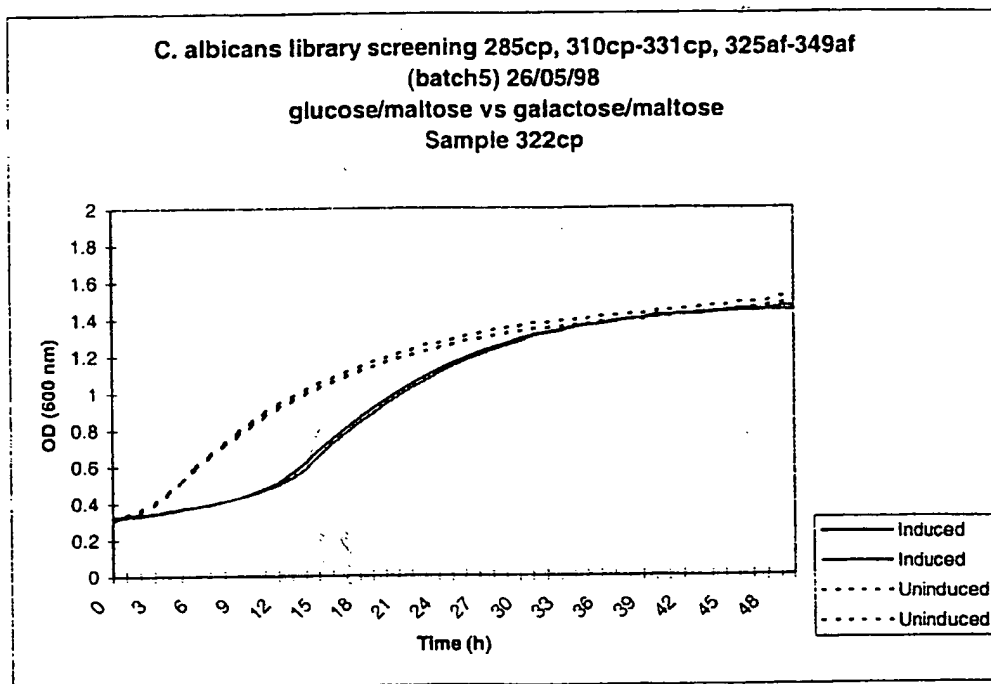
Project : Identification of novel essential genes in *C. albicans*

Strain no. : 322c_cp

Freezer location : glycerol stocks box XIV; D6

Growth curve(s) (Bioscreen) :

Date : 26/05/1998



Plasmid/clone name* : 322c_cp

Freezer location : original stocks box

Identifier (gene name) :

HTS screen :

Form generated by : Ing Loonen

(*) as it can be found in the *Candida albicans* Access dbase

33/53

FIG. 23.

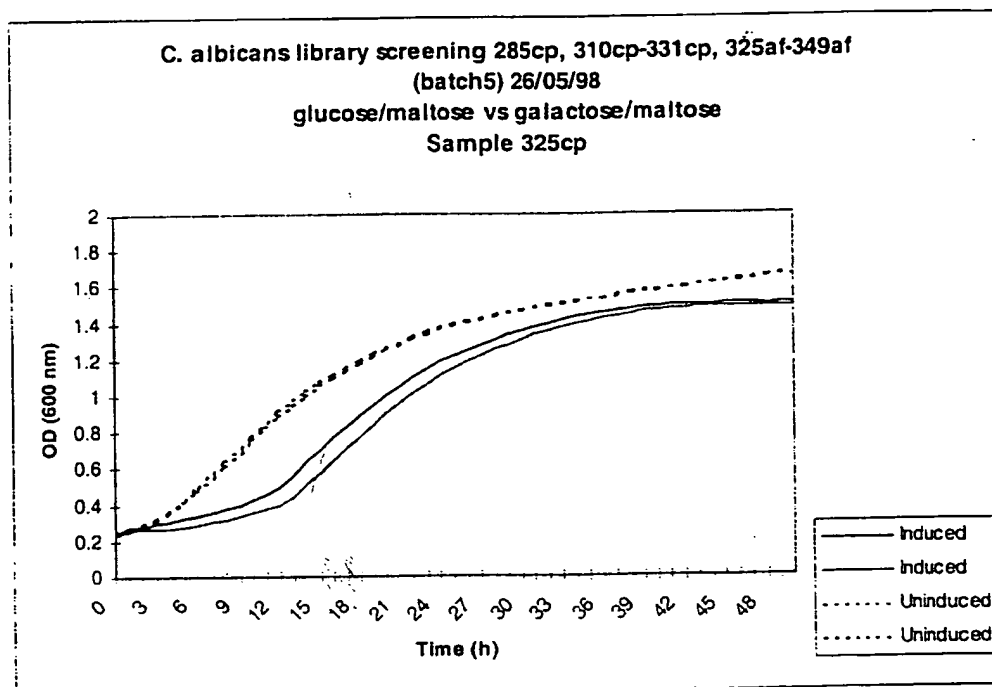
Project : Identification of novel essential genes in *C. albicans*

Strain no. : 325c_af

Freezer location : glycerol stocks box XIII; G4

Growth curve(s) (Bioscreen) :

Date : 26/05/1998



Plasmid/clone name* : 325c_af

Freezer location : original stocks box

Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the *Candida albicans* Access dbase

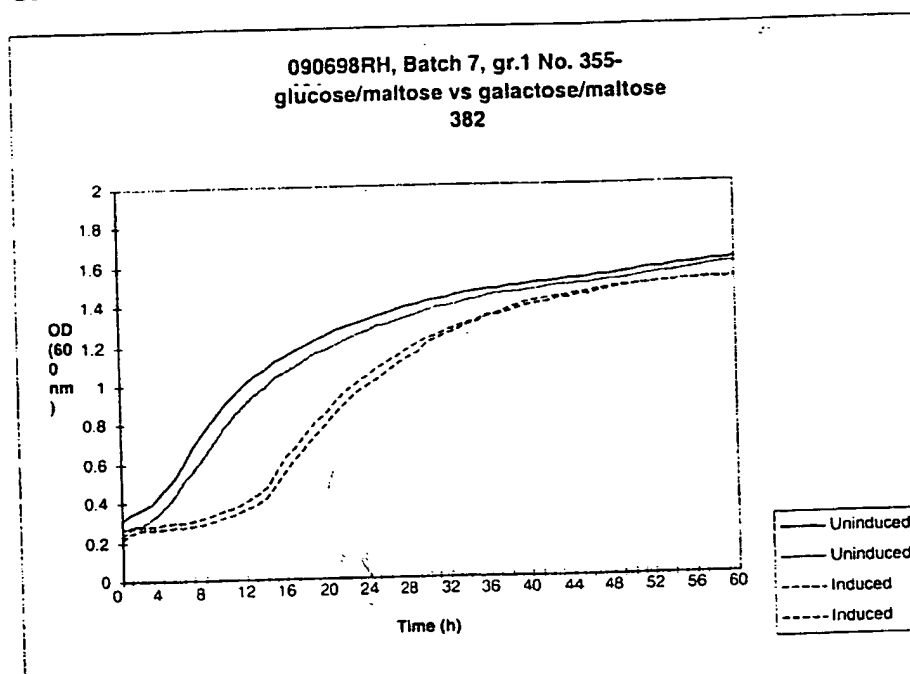
34/53

FIG. 24.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 382cp (FACS, batch 7, G1)
Freezer location : glycerol stocks box XVI; A2
Growth curve(s) (Bioscreen) :

Date : 09/06/98



Plasmid/clone name* : 382cp (purified PCR product)
Freezer location : original stocks box VIII; AAH8
Identifier (gene name) : OST4

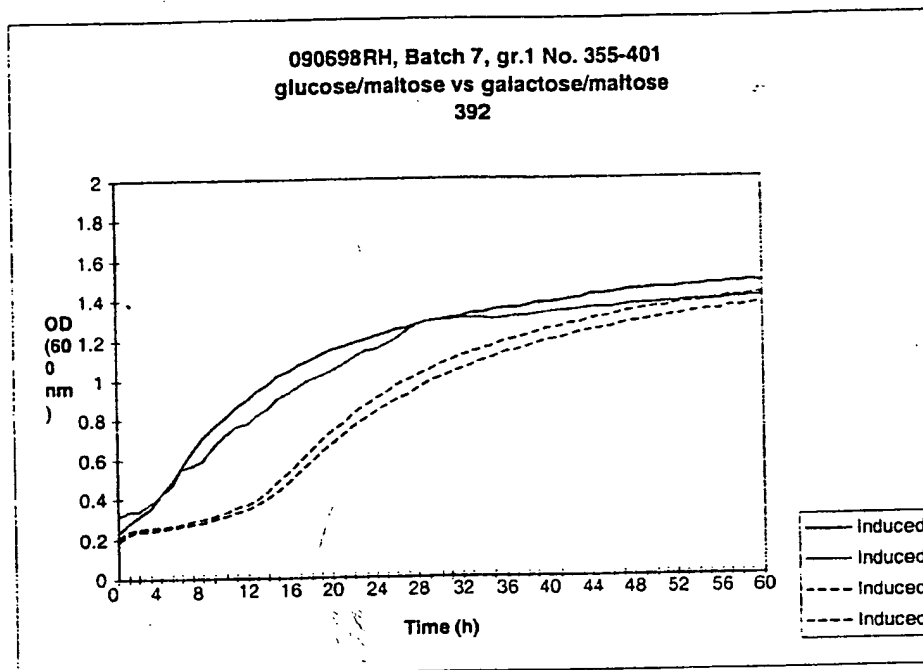
HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the *Candida albicans* Access dbase

35/53

FIG. 25.

Project : Identification of novel essential genes in *C. albicans***Strain no. : 392cp (FACS, batch 7, G1)****Freezer location : glycerol stocks box XVI; B3****Growth curve(s) (Bioscreen) :****Date : 09/06/98****Plasmid/clone name* : 392cp (purified PCR product)****Freezer location : original stocks box VIII; AAH2****Identifier (gene name) : TUF1****HTS screen :****Form generated by : Inge Lønnen****(*) as it can be found in the *Candida albicans* Access database**

36/53

FIG. 26.

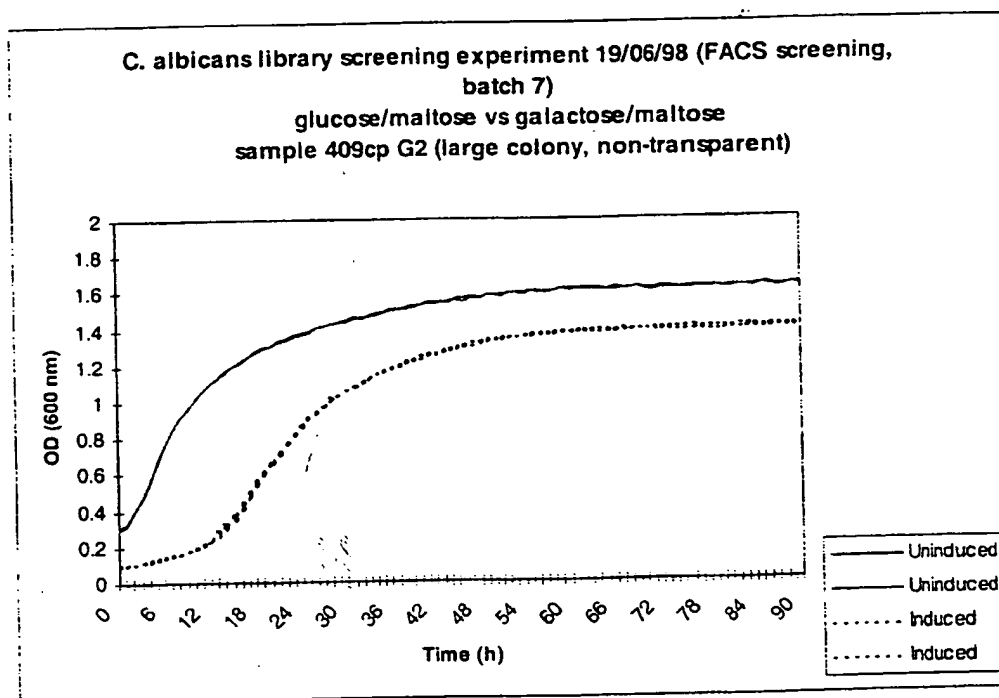
Project : Identification of novel essential genes in *C. albicans*

Strain no. : 409c_cp

Freezer location : glycerol stocks box XVI; C9

Growth curve(s) (Bioscreen) :

Date : 19/06/1998



Plasmid/clone name* : 409c_cp

Freezer location : original stocks box

Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the *Candida albicans* Access dbase

37/53

FIG. 27.

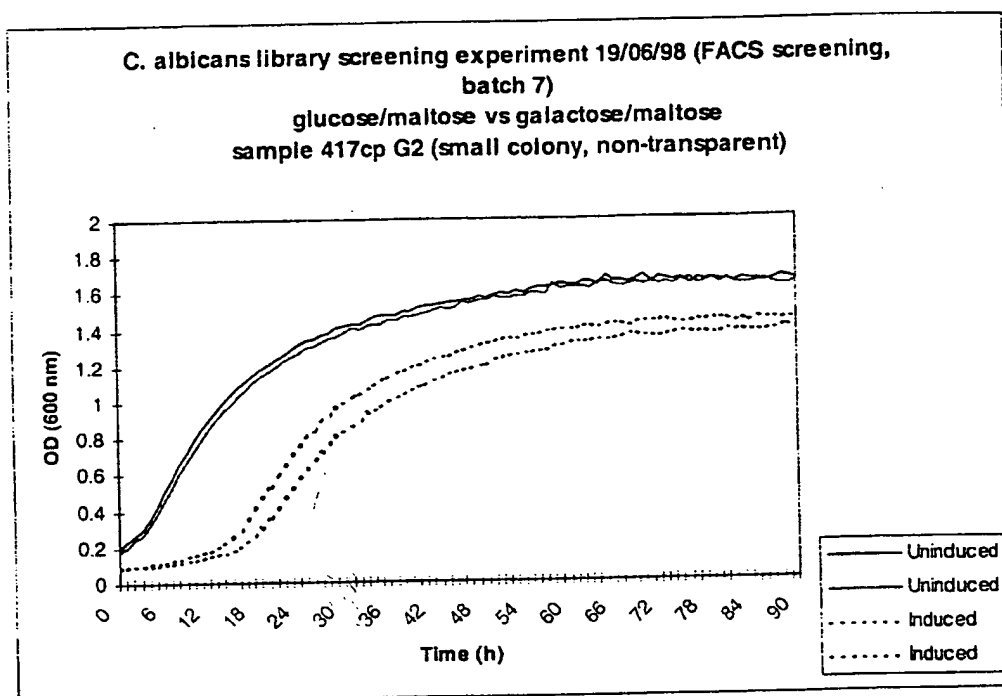
Project : Identification of novel essential genes in *C. albicans*

Strain no. : 417c_cpG2

Freezer location : glycerol stocks box XVI; D8

Growth curve(s) (Bioscreen) :

Date : 19/06/1998



Plasmid/clone name* : 417c_cpG2L

Freezer location : original stocks box

Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the *Candida albicans* Access dbase

38/53

FIG. 28.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : 325caf

Disruptant strain :

Host strain :

Freezer location :

Disruption plasmid name* :

Freezer location :

Knock-out (single/double):

Lab book ref. :

Southern results :

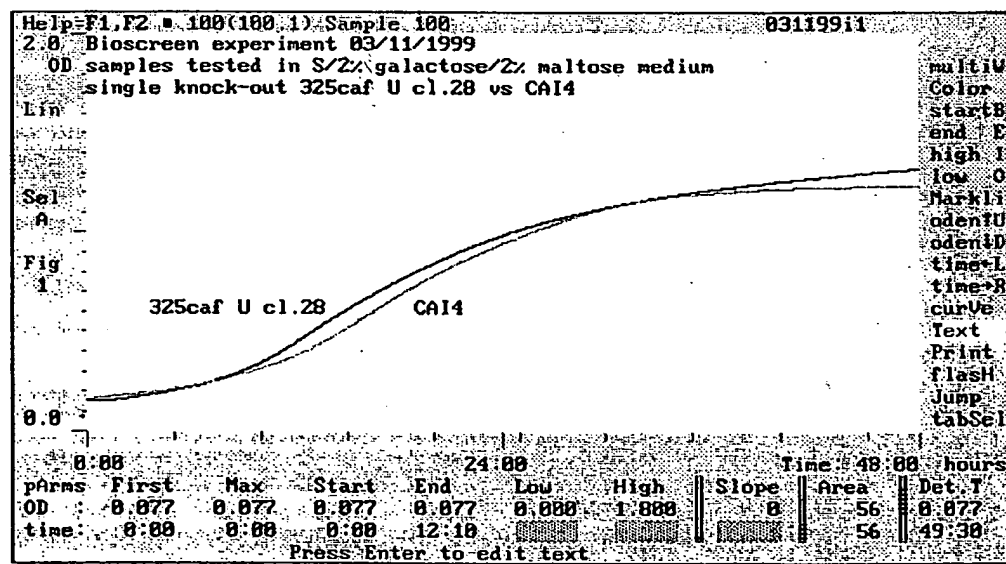
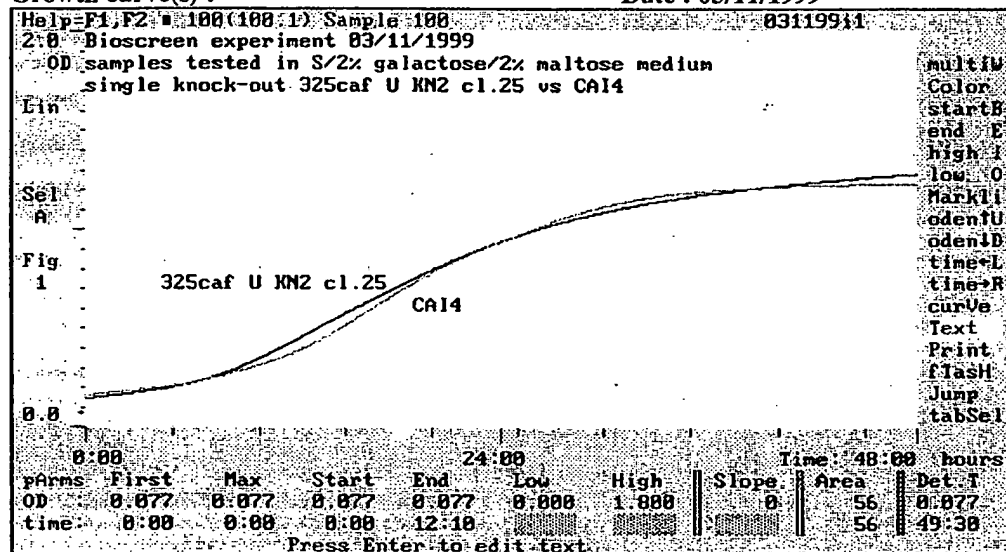
PCR results :

39/53

FIG. 28(CONTINUED 1)

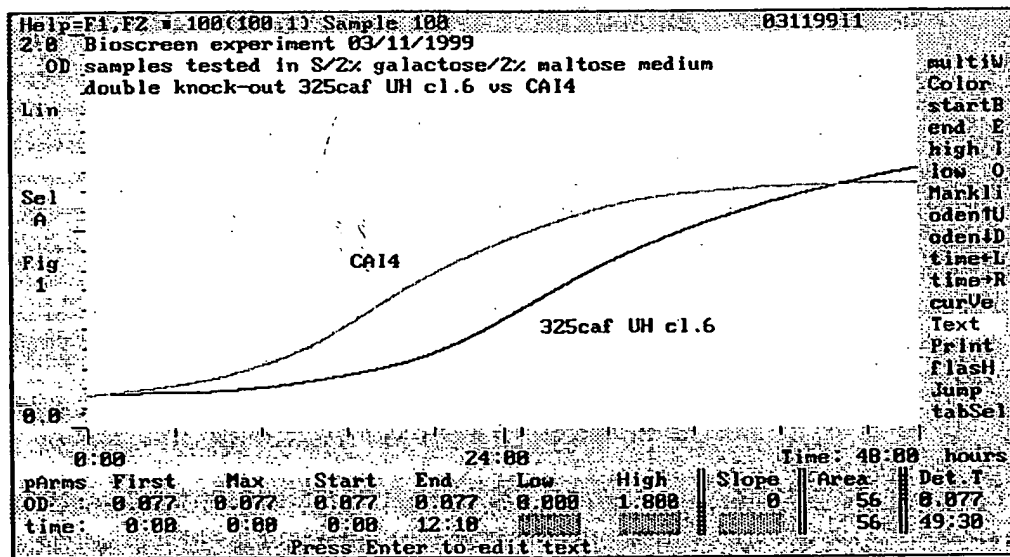
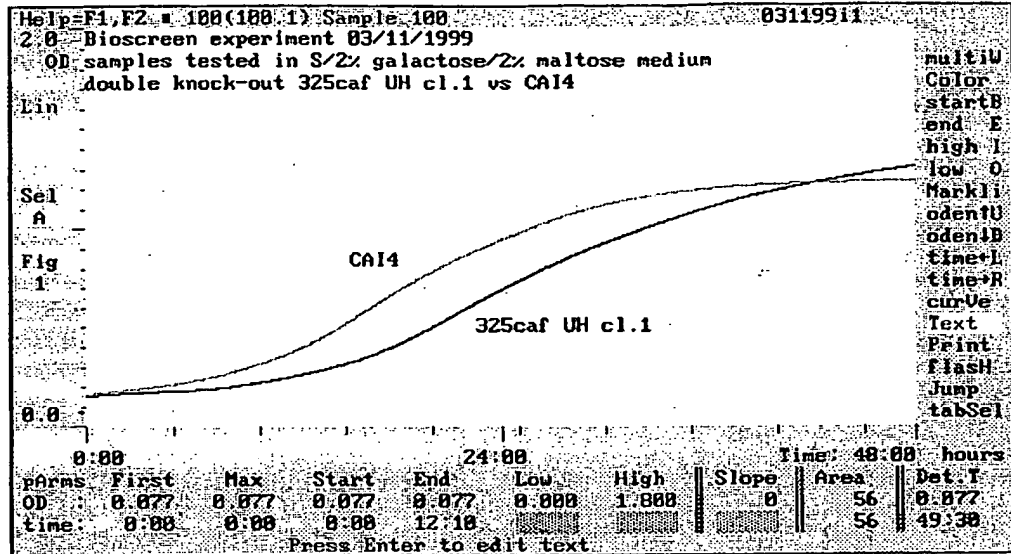
Growth curve(s) :

Date : 03/11/1999



40/53

FIG. 28 (CONTINUED 2)

**HTS screen :**

Bioscreen test of 325caf knock-out and WT growth in presence of hygromycin B

dilutions prepared

Stock solution of 53 mg/ml was prepared for hygromycin B.

From this solution dilutions were prepared of:

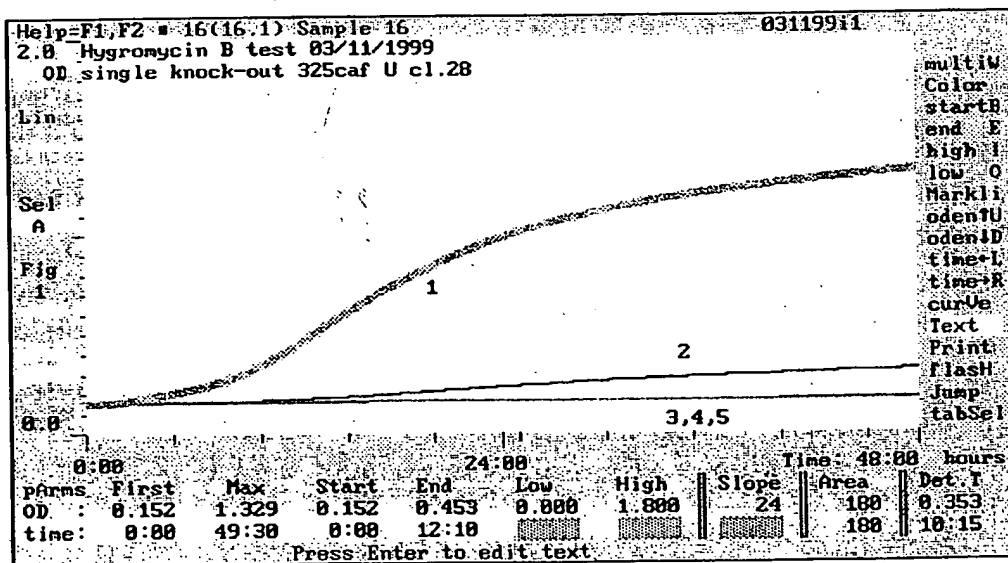
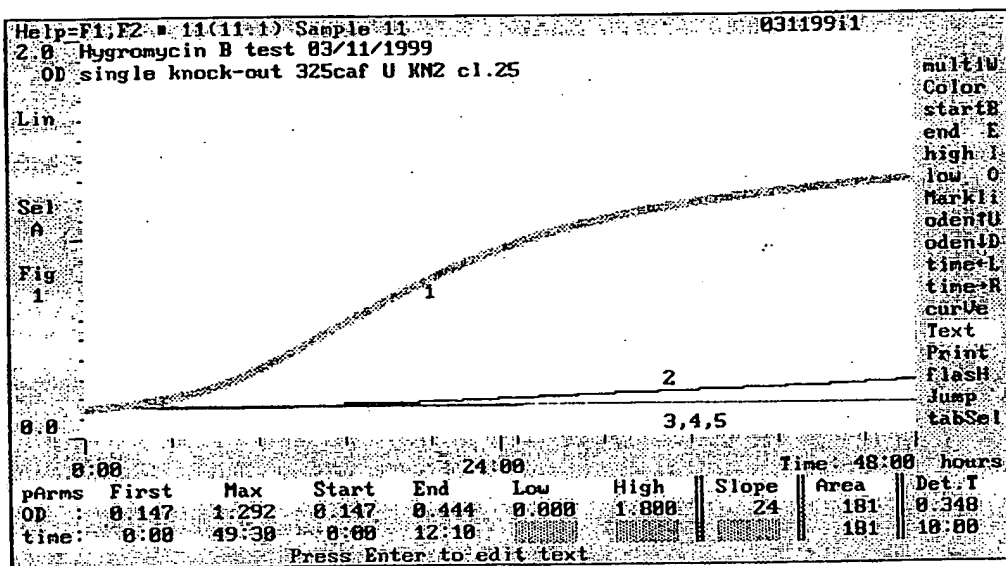
4000µg/ml, 3000µg/ml, 2000µg/ml and 1000µg/ml

Growth curves for 325cafGK knock-out and WT in the presence of hygromycin B

SUBSTITUTE SHEET (RULE 26)

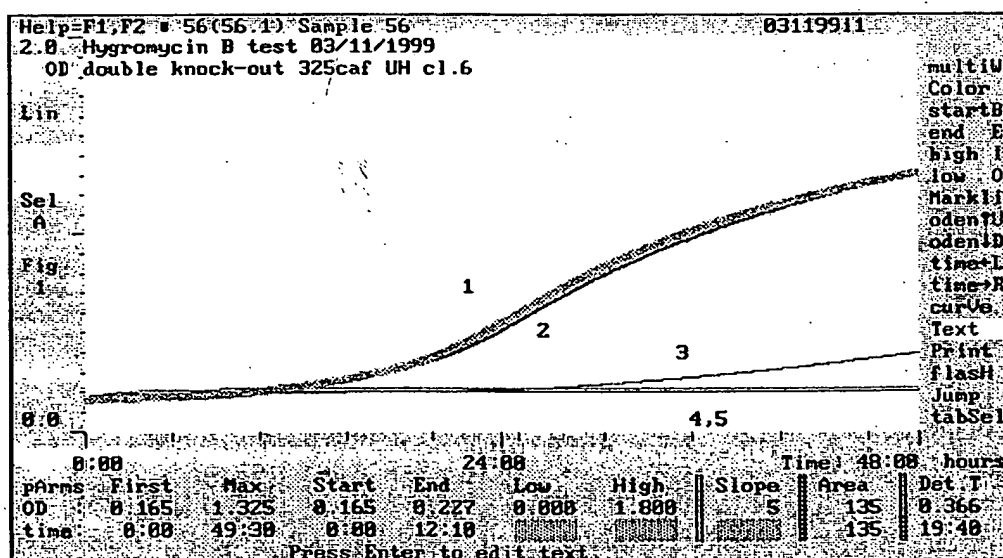
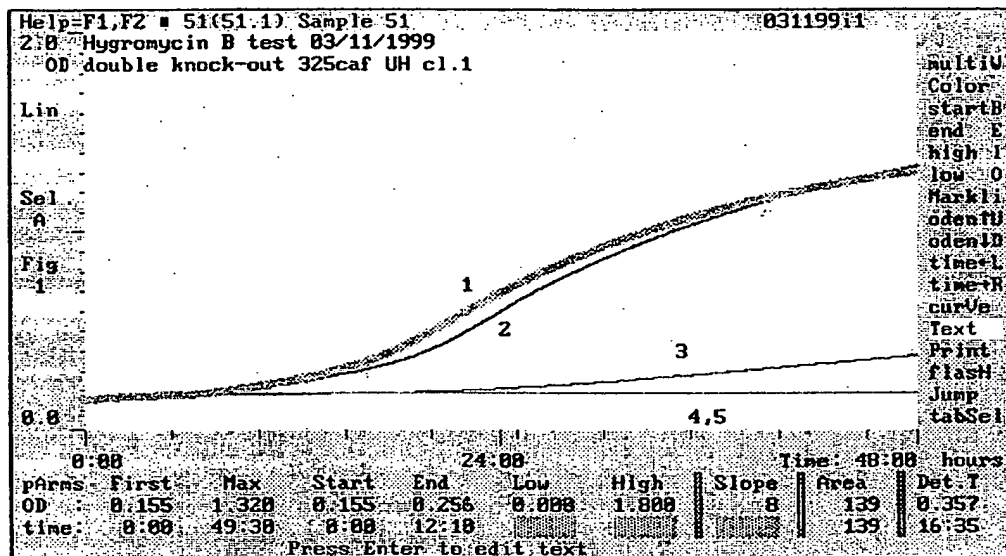
41/53

FIG. 28 (CONTINUED 3).



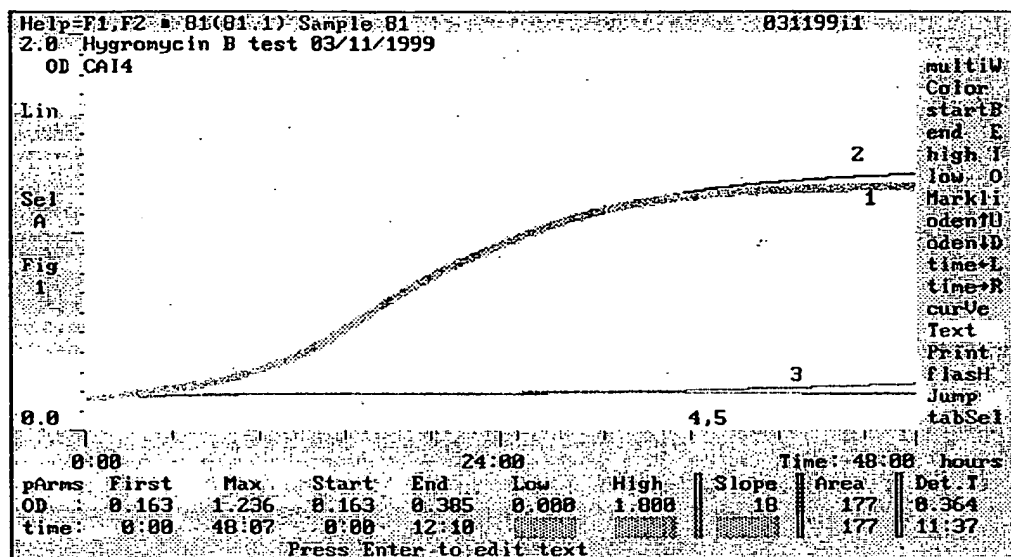
42/53

FIG. 28 (CONTINUED 4)



43/53

FIG. 28 (CONTINUED 5).

**Legend:**

- 1: S/2% gal/2% mal medium containing 0 µg/ml Hygromycin B
- 2: S/2% gal/2% mal medium containing 1000 µg/ml Hygromycin B
- 3: S/2% gal/2% mal medium containing 2000 µg/ml Hygromycin B
- 4: S/2% gal/2% mal medium containing 3000 µg/ml Hygromycin B
- 5: S/2% gal/2% mal medium containing 4000 µg/ml Hygromycin B

Form generated by :

(*) as it can be found in the Plasmid Access dbase

44/53

FIG. 29.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : 322c_cp
Disruptant strain : 322c_cp (in progress)
Host strain : CAI4NG
Freezer location : Knockout strain, box, pos.

Disruption plasmid name* : 322c_cpURAcass.(inv)/pCR2.1(inv)
Freezer location : -

Knock-out (single/double): single (in progress)
Lab book ref. : Labbook 104 of Ronald de Hoogt

Southern results :

PCR results :

45/53

FIG. 30.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : 417c_cpG2

Disruptant strain :

Host strain :

Freezer location :

Disruption plasmid name* :

Freezer location :

Knock-out (single/double):

Lab book ref. :

Southern results :

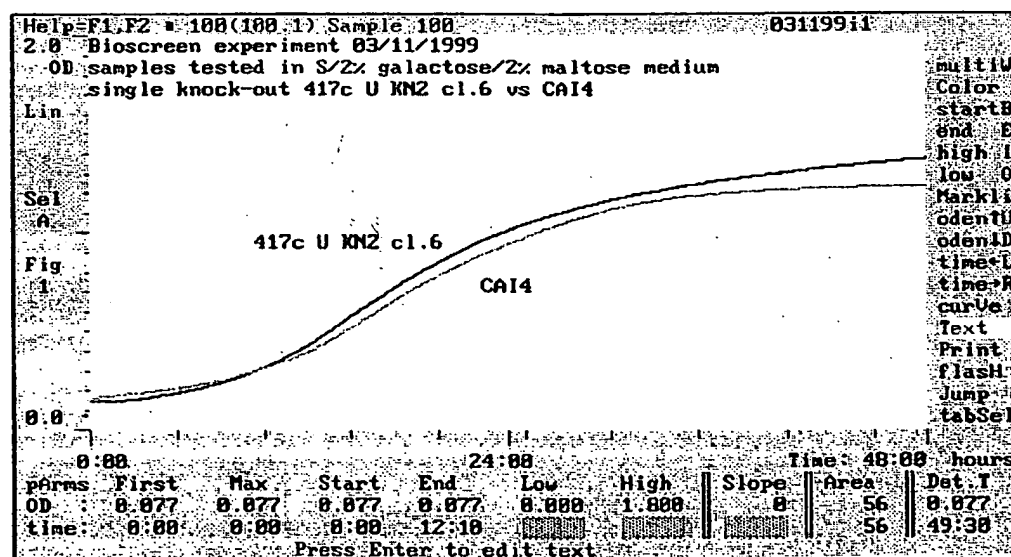
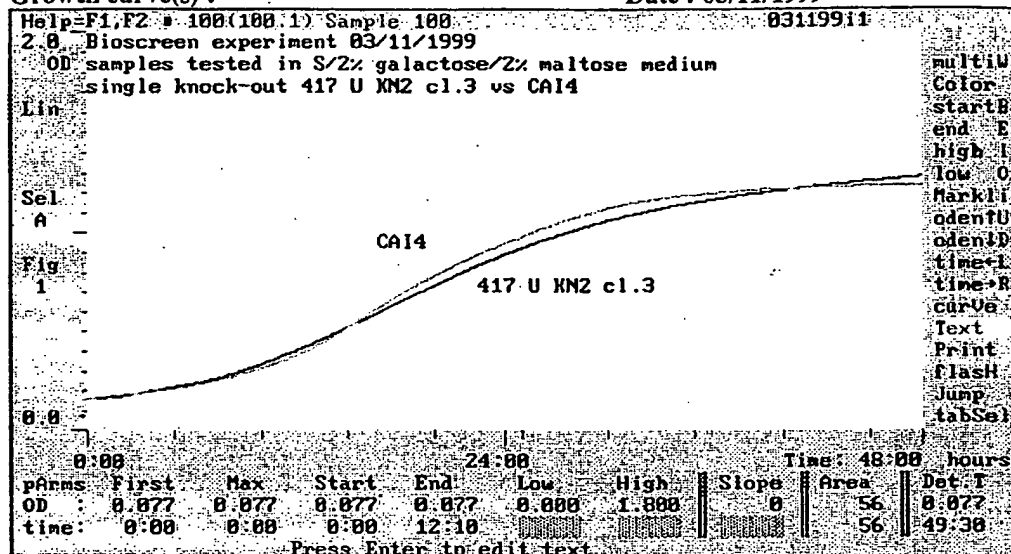
PCR results :

46/53

FIG. 30 (CONTINUED 1).

Growth curve(s) :

Date : 03/11/1999



HTS screen :

Bioscreen test of 417c_cp knock-out and WT growth in presence of hygromycin B

dilutions prepared

Stock solution of 53 mg/ml was prepared for hygromycin B.

From this solution dilutions were prepared of:

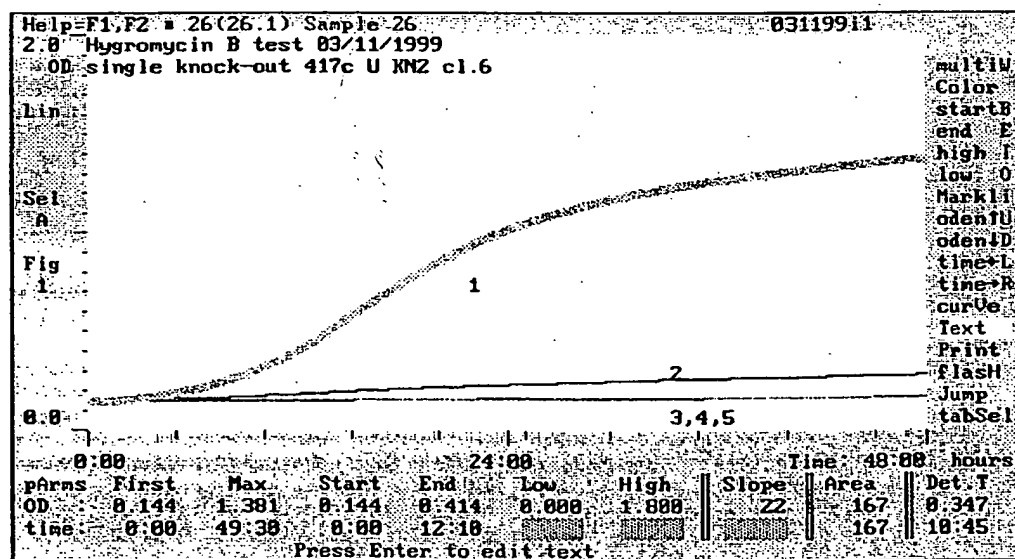
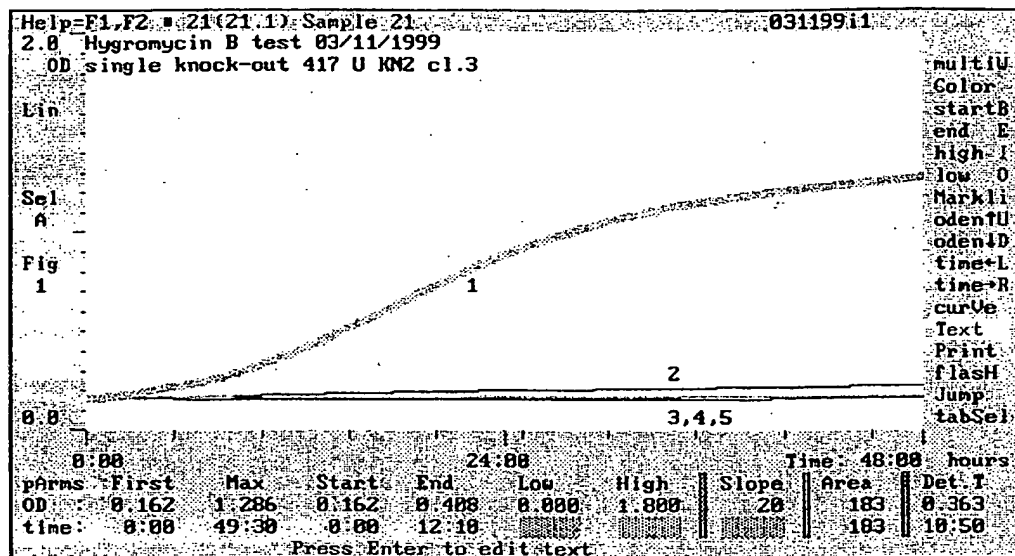
4000µg/ml, 3000µg/ml, 2000µg/ml and 1000µg/ml

Gr with curves for 417c_cp knock-out and WT in the presence of hygromycin B

SUBSTITUTE SHEET (RULE 26)

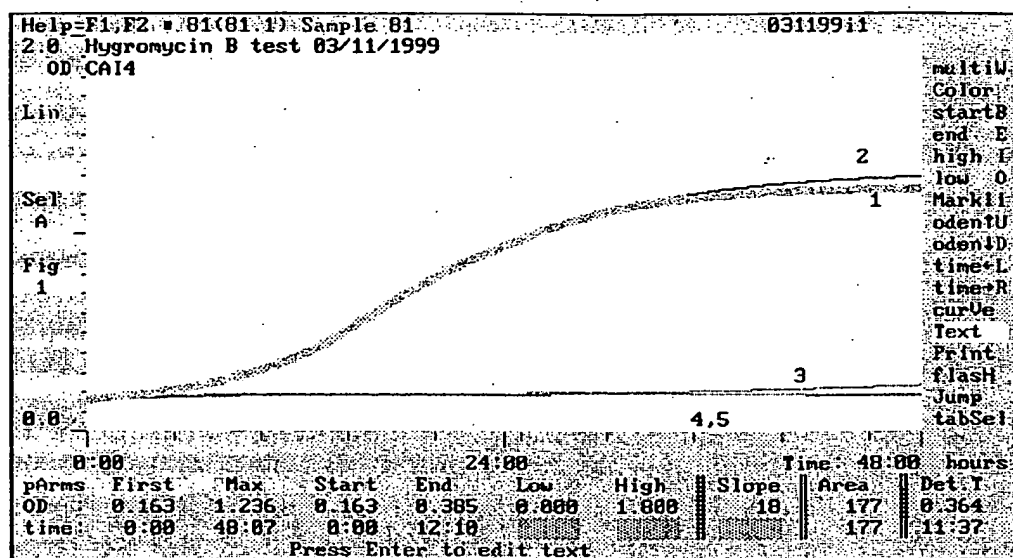
47/53

FIG. 30 (CONTINUED 2).



48/53

FIG. 30 (CONTINUED 3).

**Legend:**

- 1: S/2% gal/2% mal medium containing 0 µg/ml Hygromycin B
- 2: S/2% gal/2% mal medium containing 1000 µg/ml Hygromycin B
- 3: S/2% gal/2% mal medium containing 2000 µg/ml Hygromycin B
- 4: S/2% gal/2% mal medium containing 3000 µg/ml Hygromycin B
- 5: S/2% gal/2% mal medium containing 4000 µg/ml Hygromycin B

Form generated by :

49/53

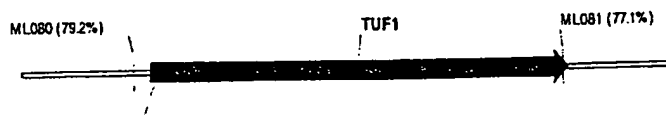
FIG. 31.

Project : Identification of novel essential genes in *C. albicans***Identifier (gene name) :** TUF1**Disruptant strain :** TUF1SAKO 7**Host strain :** CAI4/NG**Freezer location :** Strain collection Roland Contreras. YA132

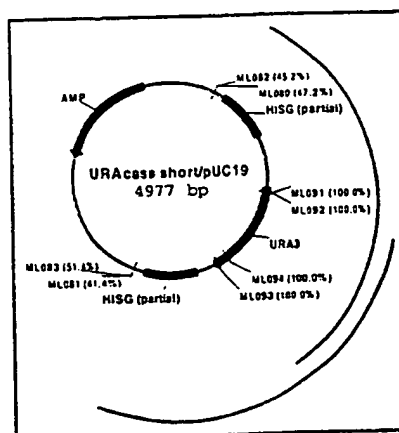
Disruption plasmid name* : Not applicable: short terminal homology (STH) PCR with overlapping fragments (split marker).

ML080	acataatcaagtgaatttacttacatcatttattgtggaatttctgaatGTGCTGGAATTCGCCCTTTATG
ML081	tcacctatataacctctttttctttttattattcacagtcacattctgtCCGGCTCGTATGTTGTG TGG
ML094	CCAGTGCTAACAACTTCATCAACAGTT
ML092	GCCTCACCAGTAGCACAAACG

Uppercase sequences are segments that anneal to the template DNA URAcass short/pUC19; the lowercase sequences are 50 nt upstream (ML080), resp. downstream (ML081) of the target ORF.



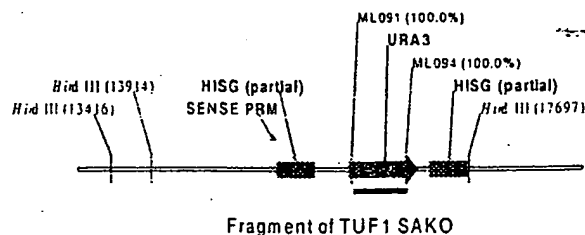
Amplification of STH fragments for TUF1 (ML080/ML094 \Rightarrow TUF1_STH-5'URA3 and ML081/ML092 \Rightarrow TUF1_STH-3'URA3). TUF1_STH-5'URA3 has a 50 bp terminal homology region upstream of the TUF1 ORF and a 3' incomplete URA3 marker; while TUF1_STH-3'URA3 has a 50 bp terminal homology region downstream of the TUF1 ORF and a 5' incomplete URA3 marker. *In vivo*, only an intact URAblast cassette can be formed when recombination occurs between the overlapping truncated URA3 sequences of the respective STH fragments.



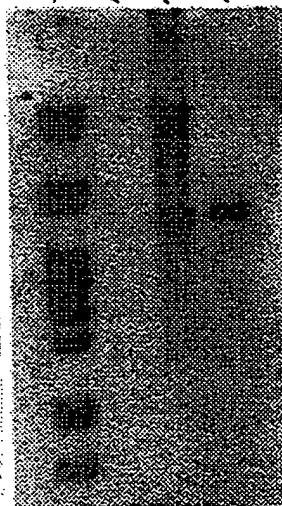
Southern results:

FIG. 31 (CONTINUED 1).

Presentation of disrupted allele



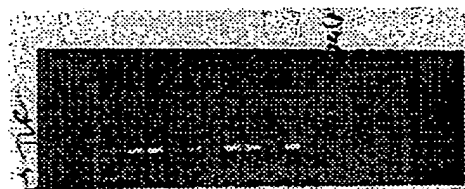
λ PstI
WT
TUF1 SAKO 6
TUF1 SAKO 7



HindIII digest
URA3 probe
Expected band: 3783 bp

PCR results:

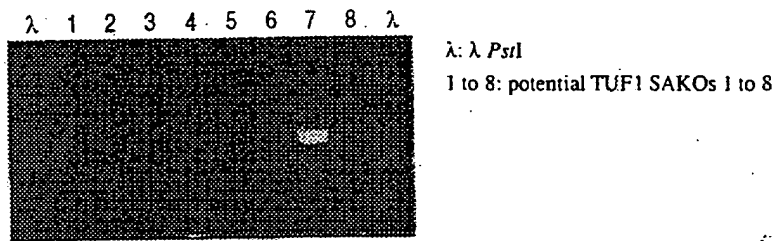
- PCR analysis was performed using the primer combination ML091/ML093 (see figure), amplifying a URA3 fragment. Band of 755 bp points to correct homologous recombination of URA3 overlapping fragments. TUF1 SAKO 7 is clearly positive (SAKO stands for single allele knock out).



lane 1: λ PstI
lane 2 to 9: potential TUF1 SAKOs 1 to 8
lane 10: Uracass short/pUC19 (positive control)
lane 11: water
lane 12: CAI4

51/53
FIG. 31(CONTINUED 2).

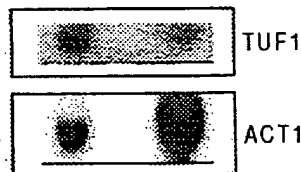
- > To check correct integration into the genome, PCR was performed with primer sets ML090/ML097. For TUF1 SAKO 7 a clear signal was obtained of the correct length of 1825 bp.



Northern analysis:

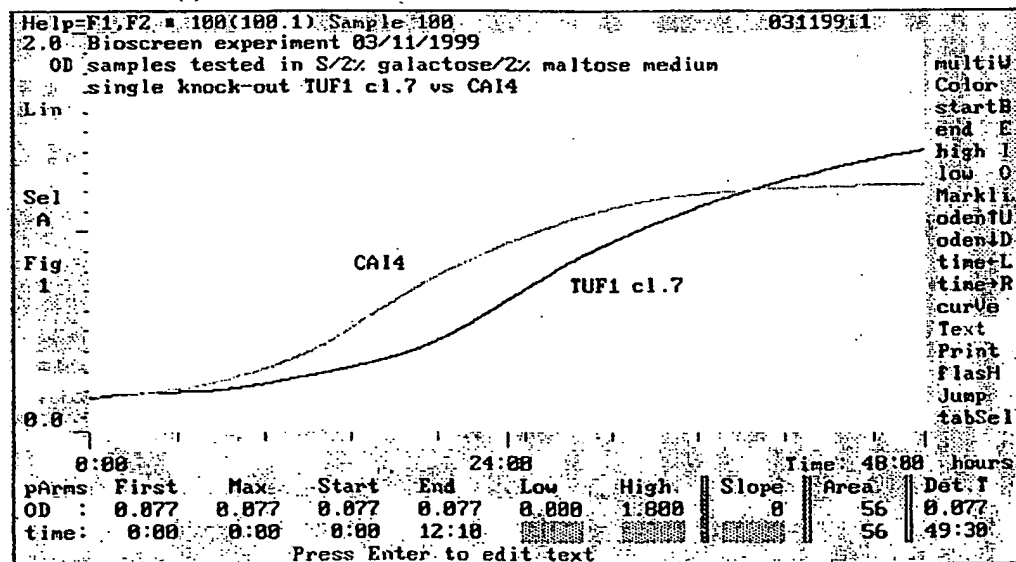
88% inhibition

TUF1 SAKO 7



Growth curve(s) :

Date : 03/11/1999



HTS screen :

FIG. 31 (CONTINUED 3)

Bioscreen test of TUF1 knock-out clone 7 and WT growth in presence of hygromycin B

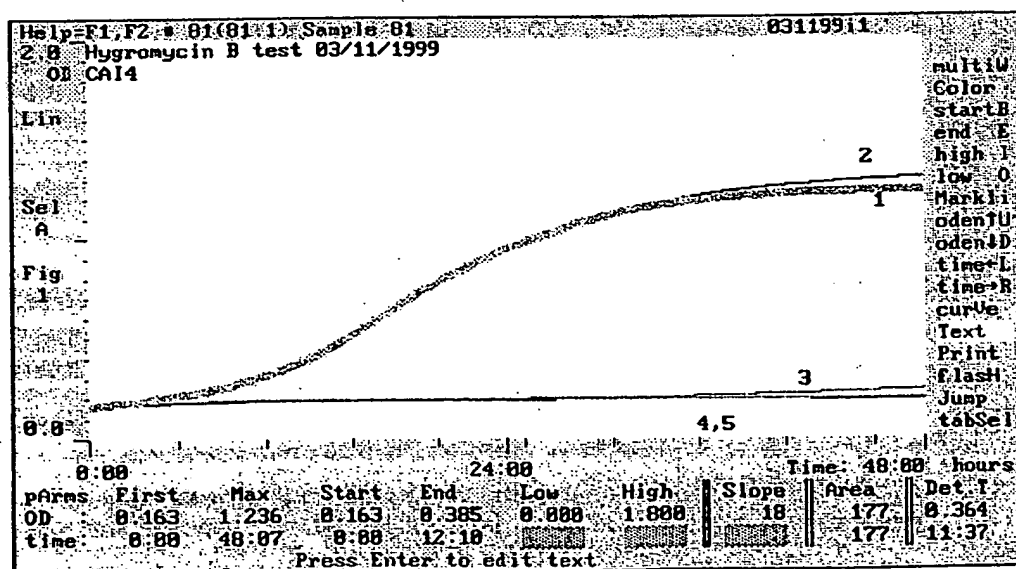
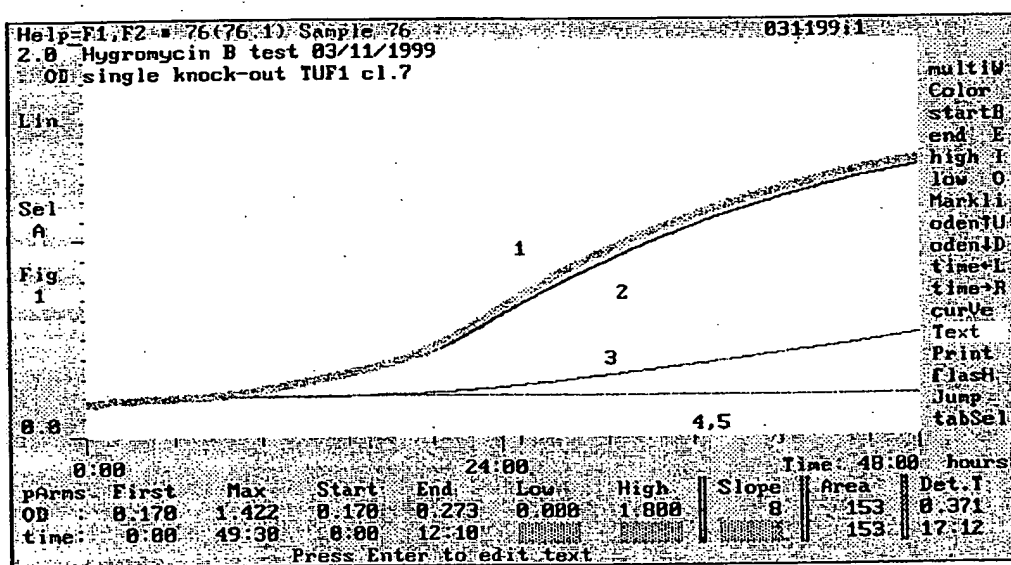
dilutions prepared

Stock solution of 53 mg/ml was prepared for hygromycin B.

From this solution dilutions were prepared of:

4000µg/ml, 3000µg/ml, 2000µg/ml and 1000µg/ml

Growth curves for TUF1 knock-out and WT in the presence of hygromycin B



Legend:

*53/53**FIG. 31 (CONTINUED 4).*

- 1: S/2% gal/2% mal medium containing 0 µg/ml Hygromycin B
- 2: S/2% gal/2% mal medium containing 1000 µg/ml Hygromycin B
- 3: S/2% gal/2% mal medium containing 2000 µg/ml Hygromycin B
- 4: S/2% gal/2% mal medium containing 3000 µg/ml Hygromycin B
- 5: S/2% gal/2% mal medium containing 4000 µg/ml Hygromycin B

SEQUENCE LISTING

<110> Janssen Pharmaceutica N. V.

<120> Drug Targets in Candida Albicans

<130> 53731/000

<140>

<141>

<150> 982204122.0

<151> 1998-12-04

<160> 16

<170> PatentIn Ver. 2.0

<210> 1

<211> 438

<212> DNA

<213> Candida albicans

<400> 1

```
aacagctggt cttctgctaa tacattcaac cctttccata tctatactcc aacaatatga 60
taactgatga acaattgaat accattgcat tgacatttgg ttttgcttca ataataattaa 120
tcataatata tcatgccata tctactaatg tacataaatt agaagatgaa accccatcat 180
cttcatttac cagaacaaat actactgaaa ctactgttgc aagtaagaaa aagaagtaat 240
aactgatgga tttttcttcc taccaccaat tgaataatgc tagacttggt ggtgtgctac 300
aaatatttca aaagaaaata cgaatacttt ataaaatggt aagaacggaa gatgggttct 360
catttataca ctaaatacaa atcacatada catcacaaaa cacaataca tacatacacc 420
tatatccctt tatttgat 438
```

<210> 2

<211> 1380

<212> DNA

<213> Candida albicans

<400> 2

```
atgttaaaaa cactaactca aactttacgc ttaactggga aagctttccc aaaggtccgt 60
ccggccttga tcagaacctc cgctgccttc gaccgttcta aacctcatgt caacattggt 120
actattgggtc atgttgatca tggtaaaact acattgactg ctgctatcac caaagtttta 180
gccgaacaag gtgggtgcaa cttcttggat tatgggtcta ttgatagagc tccagaagaa 240
agagctagag gtatcactat ttccactgcc cacgttgaat acgaaaccaa gaacagacac 300
tatgcccacg ttgattgtcc aggacacgct gattatatca aaaatatgat tactgggtgcc 360
gctcaaattg atggtgctat cattgttggt gctgccactg atggtcaaat gcctcaaacc 420
agagaacatt tgttattggc cagacaagtt ggtgttcaag acttggttgt gtttgtcaac 480
aaagtcgata ctattgatga cctgaaatg ttggaattag tcgaaatgga aatgagagaa 540
```

ttgttatcca cctacggttt tgatggtag aacactccag ttattatggg atctgcttta 600
 atggcttttg aagacaagaa accagaaatt ggtaaggaag ctatcttgaa attgtagat 660
 gctgctgatg aacacattcc aactccatca agagacttg aacaaccatt ttgttacca 720
 gttgaagacg tggtctccat ctccggtaga ggaactgttg tcaactggtag agtgaaaga 780
 ggtgttttga agaagggtag agaaatcgaa attgttggtg gttttgacaa accttacaag 840
 actactgtta ccggtattga aatgttcaaa aaagaattag actctgctat ggctggtag 900
 aactgtgggtg ttttgtaag aggtgttaaa agagatgaaa tcaagagagg tatgttttg 960
 gccaaaccag gtactgctac ttctcacaag aagttcttg cttccttgta tattttgact 1020
 tccgaagaag gtggctgctc cactccattt ggtgaagggt acaagcctca atgcttcttc 1080
 agaactaacg atgtcactac cacattttca ttcccagaag gagaagggtg tgatcattct 1140
 caaatgatca tgccaggtag caacattgaa atgggttggtg aattgatcaa atctgtcca 1200
 ttagaagtca accaacgttt caacttgaga gaagggtgta aaactgttg tactggtttg 1260
 attaccagaa tcacgaata aacagaatgt gcaactgtgaa taataaaaag aaaagaggta 1320
 tatatagggtg actttgtatt ttgtattgaa caataaaaatt ctgtaaatag taagggcctc 1380

<210> 3

<211> 2283

<212> DNA

<213> Candida albicans

<400> 3

gaattcgccc ttaagcactc gtttcaacta tacattcagt aacaacaccc ttaatttacc 60
 aaactacatt aatggaagta acacaacgga cgcagagtca gacacaacca acacaacagt 120
 caccgacaac tcagacgcaa acccaaagca aagaggacca gaataggatt tgtcaattga 180
 tttgctccac gggtcagttt ggcaattatg atttgaatat caacgataaa actatcgtag 240
 aaggtaaaat gacgtggtag tttggaagag accccaactc agatttgcaa gtggcgctcg 300
 cgtagagaat ttcaaacaag cattttcaaa tctggctcaa cttcaatgat aaatcactat 360
 ggataaagga cacttcaact aacgggacac accttaacaa cagtcgattg gtgaaaggat 420
 caaactacct tcttaatcag ggtgatgaaa tagcagtagg ggttggtaga gacgaggacg 480
 ttgtgagggt tgctgttgac tttggtgaca aatacaaccc ggcaaagcta cctgattcga 540
 ccaacacaat taaagatgaa ggaatatata aagactttat tgtgaaaaat gaaacgatag 600
 gccaaaggagc atttgccact gtgaaaaagg cgattgaacg atctacgggc gagtcgtacg 660
 cggatgaagat tataaatcga agaaaagcat taaataccgg tgggtggaagt gccatggcag 720
 gaggtagaccg tgaattgtcc atatttagagc ggctcaacca cccaaatata gttgctctaa 780
 aagcttttta tgaagatatg gacaattact atattgtgat ggaattggtg ccgggcggtg 840
 atttgatgga ctttgtggct gcaaacggtg caataggaga agacgcaaca caagtgatca 900
 cgaaacagat tctagaagga attgcctatg ttcataattt aggaatctcc catcgtgatt 960
 tgaagccaga taatattttg attatgcaag atgaccaat acttgtaaaa atcaccgact 1020
 ttggattggc aaaattcagt gacaatctga cgtttatgaa aactttttgt ggtacatttg 1080
 cgtatgttgc tcccgaagtt atcaccggtg agtatggatc atcgcatag gaactgcaac 1140
 aaaaggacaa ctactcttcc ttggttgaca tttggtcttt gggatgtttg gtttatgtac 1200
 ttttaacttc tcatttacca ttcaacggga aaaaccagca acaaattgtt gccaatgata 1260
 aaaggggcga atttcatgag gctccattaa attcatacga ctttctgaa gacggaagag 1320
 atttcttgca gtgctgccta caggtaatac ctaactaag gatgacggct gctgaagctt 1380
 tgaacataa atgggtgcaa gacttgtag aagaggattc tgtcaaatca ttgagtttat 1440
 cgcaatcaca gtcgcaacaa tctcgaaaga tagataatgg tatccatata gaatcattga 1500
 gcaaaattga tgaagacgtt atgcttcgtc cattggatag cgaaagaaat aggaaatcaa 1560
 gtaaacagca agatttcaag gtacccaagc gtgtgattcc gttatctcaa catcctgcaa 1620

```

caccgttacc aatgtcacaa ccgaaaaaga ggccgtatca aatagaccct agaacaaca 1680
aaaaagtcga tttggaagaa cctctgacaa gcaagaaagt caagctaagt gattccgttg 1740
ttgcggaaga ctacttgaag ttggggccac ttgcaaattc gttattccaa gaaacaataa 1800
atatttcaaa gtccccgttt tctttcggaa gaaatgacac ttgtgattgc gagatagacg 1860
acgacagact atccaaactt cattgtgtca ttaccaaaga aaacgactct atatggttat 1920
tggaataagag tactaactcg tgcttggtca acaatactag tgttgaaaaa ggcaacaaag 1980
ttttgcttag aggaggggag atattacatc tcttctttga ccattgtca ctgcāācata 2040
taggtttcaa agtagtcctt gttgatcaac tgtctggtga acataagagt caagtggagg 2100
ttttgaaaca aacctcagaa gaaatgaata ttattccact tatttctggt ttaagtagta 2160
taagttcata gatttagcat atatacagc atttctata gaaacaaagg ttcattaatt 2220
tagttattta cctccatgca attacattta cttcttcttc caagggcgaa ttctgcagat 2280
atc 2283

```

<210> 4

<211> 826

<212> DNA

<213> Candida albicans

<400> 4

```

atgggtagta tgtgaagata caatattgaa agtgtttact agaatatcta agatgtttga 60
gcccattggac atttttggat ttgataatta aaaaaagtag caatagatta ttgcgttgga 120
gaaagaatca ccatagttgc aagatttgat agatgttaaa atgttcacgc aggcgaaaga 180
tgtaacatct cttaaagtaa gaagaatatg gacatgaata aaaatagata gcactatttt 240
ggaacttggt gaagatatta aaatagaatg ggatttcaac atagatattc aaagtaacga 300
aacctcacia tcaataaaaa acaacagtaa tactaacaat tcaattttta tttttataga 360
gggtactcca tctttaggta aacgtcacia caaatctcac accttatgta acagatgtgg 420
ccgtcgttca ttccacgtcc aaaagaagac ctgttcttct tgtggttacc cagctgctaa 480
aatgagatct cacaactggg ctttaaaagc caaagaaga agaactactg gtaccggtag 540
aatggcttac ttgaacacg ttaccagaag attcaagaac ggtttcctaaa ctgggtgtgc 600
taaagctcaa accccttccg ctttaactaa ttactgaagt tattgggtcat gcattagtca 660
ttattcatta aagtcattgt aagcātagca aaggaagaat tggttagatt cttgtttaaa 720
atgtaatgac tatttaatat ctgtttaaat aagagggtta gtctttattt ttttacgtat 780
acaccaaaaa aaaaagaac aaataaaatc tgtatattaa tggttg 826

```

<210> 5

<211> 978

<212> DNA

<213> Candida albicans

<400> 5

```

atgggtacta gtacaagtga agcattgaag aacatcaaaa acaaacagcg aagacagaaa 60
gtttttgcag aaataaaaca tgaaaagaat aaacaacgtc ataagcaaag agccgaaaga 120
gctaagggaag aaagagaaaa ccccgaatta agagaggaaa gaatagcagc taatatccca 180
gatactatag atagcaaacg tatttatgat gagactatag ctgctgaagt tgaaggagat 240
gacgagtttc agtcattatt cactaacttg ttggaagaac caaagatttt gttgacaaca 300
agtgcāatg ctaaaaaacc ggcttatgaa tttgcagaca tgatcatgga ctttttaccg 360
aatgtgacat ttatcaaaag gaagaaggaa tatacaatgc aagatatggc caaatattgc 420
tcgaatagag acctcactgc attgcttgtc atcaacgaag acaagaagaa ggtcaatggt 480

```

ataacgctca tcaatttacc tgaagggcca acattttatt tttcgattac atcaatagtt 540
 gatgggaaaa gaattaaggg acacgggaaa gctgggtgatt atttacctga gattgtattg 600
 aataatttca attcaagatt gggtaaaact gtgggaagac tatttcaaag tattttccct 660
 cataaacctg aacttcaagg aagacaagtg attactttgc acaatcaacg tgattatatt 720
 tttttcagaa gacatagata ttttttcaga aatgaggaaa aggttggatt gcaggaattg 780
 ggtccgcagt ttacattaaa gctaagaaga atgcaaaagg gagtacgtgg tgatgttgtt 840
 tgggaacaca gaccagatat ggaaagagat aagaagaagt tttatttata agcgggtgta 900
 taaaggtagt agtagtgcgt ttataagtat gtgtgtgtgt ttatgcatag atgtgtaaag 960
 agtaatacag ctaattcg 978

<210> 6

<211> 619

<212> DNA

<213> *Candida albicans*

<400> 6

aactaatttg tttaaaccatc aataccaaga agttttttaca attcaatccc acatacacca 60
 ttaattatga attctgaaaa gattattgaa gttatcattg ctattttctt accaccagta 120
 gctgtgttta tgaaatgtgg tgccactacc ccattatgga ttaacttggg attatgtatc 180
 tttatttggg tccctgctat cttacatgcc ttatacgttg tgttgaaaga ttaaacaaac 240
 accagagatt tactgcttga tgaattgatt actccaaaga gttgtgacta gttcccagtg 300
 tgtttttttt gccttccaac tttcttttac atttttccat tactaccact gtcttcccc 360
 ctattttgca gagttttcaa aatttatcca aaacatgtta gtcattaaac catattatta 420
 taattattct tttttgtatt tttttccctt aaaacacggt aatttattaa tcgtttcggt 480
 gtttggtatt ttattttttt gtatttatca attggaatat atatctatac atgaatttat 540
 tatccattgt accaattggt aaaacatttt gtagttttt tgttactagt ataaaannat 600
 aataaaagtt tanttcaac 619

<210> 7

<211> 2319

<212> DNA

<213> *Candida albicans*

<400> 7

atgacattag ggttcgataa attcataagc aagggtcagca ctcatagacg tcaatctgaa 60
 ccatcaatct tggaaatcgc agccaccaat tctcaaaata aatcgagaag gctaagtatg 120
 gataatgggc attgttatgt tcgtgaatca actaataatc atcatcattt aaataccgctc 180
 gttgataatt tacgacagcg tgccgggatcg ttttcattta tttcacatca ccataatcac 240
 catcagaata gtcacgataa ttatactgtc gatcccctta catcaaacgg agcacgaatt 300
 tcccgatcac gttcacgttc caaatcagtt gggcacggag aagcaatatc accagcgat 360
 ttttccaaga ataaaaccaa agatttagtg aaacaggaaa cagcacatat cattctgaag 420
 aaattactca acatgttaca agatttggat ttacaaaacc ctattgcatt gaaaaaata 480
 tcacaagggt cagaatcaaa gttttgtaaa atctacgtgt ctaacactaa taattgtatt 540
 tacttaccag cagcaagttc aacaagtttc acttatgaag atgatgaaa tggcggcggt 600
 ataattgctg aagatagaaa tgatgaaatg ccaacagcag ttaataacaa tactttgtca 660
 atggatagta taaatcattc agagactgat ttcttggtt ctccaccacc tccagattta 720
 ttttctaaaa tgaaatcatt ccattcacca aattacttga cttcaaaaat cgattctgaa 780
 tgtccaattc cacatacatt tgctgtgatt gttgaattaa ccaaggactc tttgattatt 840

aaagatcttc atttccaatt tcagtcatta actaccattt tatggccaac tggggatgca 900
 tataatcgga ctcatgccaa ggagaaattt accattggga atatggaatg gcgtacatct 960
 ttaagcgacg ccgactatta tatcaatagt tctaattcca acgatgttaa gctgaaaaac 1020
 ttgggtcctg aagatcttat taatcgaact agagaataca aattaatcga tattgaagaa 1080
 ccaaacaatt catcaaacag tttactggat gatgacatgg atattaataa tattacgtcg 1140
 ccattatcaa cgtcaccaac atcaagttca acttcaacaa attcaacctc caactcattg 1200
 gggttcagatt catataaagc tgggtctttat gtatttttat taccaatctt attgccagaa 1260
 catattcctg cttccattgt ttctattaat gggttcattgg ctcatacatt actgggtgaa 1320
 tgcaataaat atactgataa gttgaatcgg aaatcaaaaag tatcagcatc gtacaattta 1380
 cctatgggtcc gtactccacc aaacattgggt aattccattg ctgataagcc aatttatgtt 1440
 aataggattt ggaatgatgc cgtacattat attataactt tccccgcaa atatgttact 1500
 ttgggttggtg aacacatgat aaatgtgaaa ttactgcca tgggtgaaaga tgtggttatc 1560
 aagcgtatta aatttaatgt attggagaga ataacttatg tttccaaaaa tttatcacga 1620
 gaatatgatt atgatagtga agacccttat tgtattcatc cagtttctaa agaaaataaa 1680
 gtacgtgaac gtgttggtgc gttatatgaa ttgaaaacga aggcaaaaaca atcttctggt 1740
 ggacatcttg aagcttataa acaagaagtt atgaaatgac cggaaaataa ccttttattt 1800
 tcttggtatg aggttgaaaa tgataataat aacggcaacg gcaacggcaa cggcaacgga 1860
 aacaagaacg ttaaacaaaa gaataaagat caaccaatga ttgctacacc ttagatatc 1920
 aatgtttctt taccattttt aactactatg tctgatagtt taattatgac atcagccata 1980
 gaagaagaag gttcagatct gcctcatata tcaagaagag ggtcggcagt gagtatgact 2040
 gataataata ctacccaag taacaataac cctttatctc catttttggg agcagtggaa 2100
 actaatggtg ctagtataaa tgaaattggt gatcatatcatt tttccctga ttctaatttt 2160
 cgacatattg aaattaaaca tcgattacaa gttacattta ggatttctaa accggatctg 2220
 gataataaaa tgcattcatta tgaagtgggt attgataccc ccacgtttt acttagttca 2280
 aaatgtcaag aagattctcc tctccttat agttctgta 2319

<210> 8

<211> 255

<212> DNA

<213> Candida albicans

<400> 8

aacgttcgtg caaaaggcta tactggtgat atccacgcag atgaagagca agtttaatca 60
 actctttgtc aattaatgct gtacttggtt tcattttatt tgctggcatt taaagaatac 120
 ccatagttca gaaaataaaa ttgaaaaatt taaaaaaaaa cgcaatatca ttcatttttt 180
 ttgttttttt gacaataata ttaatatgta gttaccaatg ttttttagatt ttatatgttt 240
 tgaaaaaata gtttg 255

<210> 9

<211> 119

<212> DNA

<213> Candida albicans

<400> 9

aaccttacaa tcattatacc aactatcaaa atcataagac tcttnaactt ctgtttttga 60
 tagttggtat aatgatttat gattatctt aattcattat tattagtttc ggtcacaaa 119

<210> 10

<211> 60

<212> PRT

<213> Candida albicans

<400> 10

Met Ile Thr Asp Glu Gln Leu Asn Thr Ile Ala Leu Thr Phe Gly Phe
 1 5 10 15

Ala Ser Ile Ile Leu Ile Ile Ile Tyr His Ala Ile Ser Thr Asn Val
 20 25 30

His Lys Leu Glu Asp Glu Thr Pro Ser Ser Ser Phe Thr Arg Thr Asn
 35 40 45

Thr Thr Glu Thr Thr Val Ala Ser Lys Lys Lys Lys
 50 55 60

<210> 11

<211> 426

<212> PRT

<213> Candida albicans

<400> 11

Met Leu Lys Thr Leu Thr Gln Thr Leu Arg Leu Thr Gly Lys Ala Phe
 1 5 10 15

Pro Lys Val Arg Pro Ala Leu Ile Arg Thr Tyr Ala Ala Phe Asp Arg
 20 25 30

Ser Lys Pro His Val Asn Ile Gly Thr Ile Gly His Val Asp His Gly
 35 40 45

Lys Thr Thr Leu Thr Ala Ala Ile Thr Lys Val Leu Ala Glu Gln Gly
 50 55 60

Gly Ala Asn Phe Leu Asp Tyr Gly Ser Ile Asp Arg Ala Pro Glu Glu
 65 70 75 80

Arg Ala Arg Gly Ile Thr Ile Ser Thr Ala His Val Glu Tyr Glu Thr
 85 90 95

Lys Asn Arg His Tyr Ala His Val Asp Cys Pro Gly His Ala Asp Tyr
 100 105 110

Ile Lys Asn Met Ile Thr Gly Ala Ala Gln Met Asp Gly Ala Ile Ile
 115 120 125

Val Val Ala Ala Thr Asp Gly Gln Met Pro Gln Thr Arg Glu His Leu
 130 135 140

Leu Leu Ala Arg Gln Val Gly Val Gln Asp Leu Val Val Phe Val Asn
 145 150 155 160

Lys Val Asp Thr Ile Asp Asp Pro Glu Met Leu Glu Leu Val Glu^{Met}
 165 170 175

Glu Met Arg Glu Leu Leu Ser Thr Tyr Gly Phe Asp Gly Asp Asn Thr
 180 185 190

Pro Val Ile Met Gly Ser Ala Leu Met Ala Leu Glu Asp Lys Lys Pro
 195 200 205

Glu Ile Gly Lys Glu Ala Ile Leu Lys Leu Leu Asp Ala Val Asp Glu
 210 215 220

His Ile Pro Thr Pro Ser Arg Asp Leu Glu Gln Pro Phe Leu Leu Pro
 225 230 235 240

Val Glu Asp Val Phe Ser Ile Ser Gly Arg Gly Thr Val Val Thr Gly
 245 250 255

Arg Val Glu Arg Gly Val Leu Lys Lys Gly Glu Glu Ile Glu Ile Val
 260 265 270

Gly Gly Phe Asp Lys Pro Tyr Lys Thr Thr Val Thr Gly Ile Glu Met
 275 280 285

Phe Lys Lys Glu Leu Asp Ser Ala Met Ala Gly Asp Asn Cys Gly Val
 290 295 300

Leu Leu Arg Gly Val Lys Arg Asp Glu Ile Lys Arg Gly Met Val Leu
 305 310 315 320

Ala Lys Pro Gly Thr Ala Thr Ser His Lys Lys Phe Leu Ala Ser Leu
 325 330 335

Tyr Ile Leu Thr Ser Glu Glu Gly Gly Arg Ser Thr Pro Phe Gly Glu
 340 345 350

Gly Tyr Lys Pro Gln Cys Phe Phe Arg Thr Asn Asp Val Thr Thr Thr
 355 360 365

Phe Ser Phe Pro Glu Gly Glu Gly Val Asp His Ser Gln Met Ile Met
 370 375 380

Pro Gly Asp Asn Ile Glu Met Val Gly Glu Leu Ile Lys Ser Cys Pro
 385 390 395 400

Leu Glu Val Asn Gln Arg Phe Asn Leu Arg Glu Gly Gly Lys Thr Val
 405 410 415

Gly Thr Gly Leu Ile Thr Arg Ile Ile Glu
 420 425

<210> 12

<211> 699

<212> PRT

<213> Candida albicans

<400> 12

Met Glu Val Thr Gln Arg Thr Gln Ser Gln Thr Gln Pro Thr Gln Gln
 1 5 10 15

Ser Pro Thr Thr Gln Thr Gln Thr Gln Ser Lys Glu Asp Gln Asn Arg
 20 25 30

Ile Cys Gln Leu Ile Cys Ser Thr Gly Gln Phe Gly Asn Tyr Asp Leu
 35 40 45

Asn Ile Asn Asp Lys Thr Ile Val Gln Gly Lys Met Thr Trp Tyr Phe
 50 55 60

Gly Arg Asp Pro Asn Ser Asp Leu Gln Val Ala Ser Ser Ser Arg Ile
 65 70 75 80

Ser Asn Lys His Phe Gln Ile Trp Leu Asn Phe Asn Asp Lys Ser Leu
 85 90 95

Trp Ile Lys Asp Thr Ser Thr Asn Gly Thr His Leu Asn Asn Ser Arg
 100 105 110

Leu Val Lys Gly Ser Asn Tyr Leu Leu Asn Gln Gly Asp Glu Ile Ala
 115 120 125

Val Gly Val Gly Arg Asp Glu Asp Val Val Arg Phe Val Val Val Phe
 130 135 140

Gly Asp Lys Tyr Asn Pro Ala Lys Leu Pro Asp Ser Thr Asn Thr Ile
 145 150 155 160

Lys Asp Glu Gly Ile Tyr Lys Asp Phe Ile Val Lys Asn Glu Thr Ile
 165 170 175

Gly Gln Gly Ala Phe Ala Thr Val Lys Lys Ala Ile Glu Arg Ser Thr
 180 185 190

Gly Glu Ser Tyr Ala Val Lys Ile Ile Asn Arg Arg Lys Ala Leu Asn
 195 200 205

Thr Gly Gly Gly Ser Ala Met Ala Gly Val Asp Arg Glu Leu Ser Ile
 210 215 220

Leu Glu Arg Leu Asn His Pro Asn Ile Val Ala Leu Lys Ala Phe Tyr
 225 230 235 240

Glu Asp Met Asp Asn Tyr Tyr Ile Val Met Glu Leu Val Pro Gly Gly
 245 250 255

Asp Leu Met Asp Phe Val Ala Ala Asn Gly Ala Ile Gly Glu Asp Ala
 260 265 270

Thr Gln Val Ile Thr Lys Gln Ile Leu Glu Gly Ile Ala Tyr Val His
 275 280 285

Asn Leu Gly Ile Ser His Arg Asp Leu Lys Pro Asp Asn Ile Leu Ile
 290 295 300

Met Gln Asp Asp Pro Ile Leu Val Lys Ile Thr Asp Phe Gly Leu Ala
 305 310 315 320

Lys Phe Ser Asp Asn Ser Thr Phe Met Lys Thr Phe Cys Gly Thr Leu
 325 330 335

Ala Tyr Val Ala Pro Glu Val Ile Thr Gly Lys Tyr Gly Ser Ser Gln
 340 345 350

Met Glu Ser Gln Gln Lys Asp Asn Tyr Ser Ser Leu Val Asp Ile Trp
 355 360 365

Ser Leu Gly Cys Leu Val Tyr Val Leu Leu Thr Ser His Leu Pro Phe
 370 375 380

Asn Gly Lys Asn Gln Gln Gln Met Phe Ala Lys Ile Lys Arg Gly Glu
 385 390 395 400

Phe His Glu Ala Pro Leu Asn Ser Tyr Asp Ile Ser Glu Asp Gly Arg
 405 410 415

Asp Phe Leu Gln Cys Cys Leu Gln Val Asn Pro Lys Leu Arg Met Thr
 420 425 430

Ala Ala Glu Ala Leu Lys His Lys Trp Leu Gln Asp Leu Tyr Glu Glu
 435 440 445
 Asp Ser Val Lys Ser Leu Ser Leu Ser Gln Ser Gln Ser Gln Gln Ser
 450 455 460
 Arg Lys Ile Asp Asn Gly Ile His Ile Glu Ser Leu Ser Lys Ile Asp
 465 470 475 480
 Glu Asp Val Met Leu Arg Pro Leu Asp Ser Glu Arg Asn Arg Lys Ser
 485 490 495
 Ser Lys Gln Gln Asp Phe Lys Val Pro Lys Arg Val Ile Pro Leu Ser
 500 505 510
 Gln His Pro Ala Thr Pro Leu Pro Met Ser Gln Pro Lys Lys Arg Pro
 515 520 525
 Tyr Gln Ile Asp Pro Arg Thr Asn Lys Lys Val Asp Leu Glu Glu Pro
 530 535 540
 Ser Thr Ser Lys Lys Val Lys Leu Ser Asp Ser Val Val Ala Glu Asp
 545 550 555 560
 Tyr Leu Lys Leu Gly Pro Leu Ala Asn Ser Leu Phe Gln Glu Thr Ile
 565 570 575
 Asn Ile Ser Lys Ser Pro Phe Ser Phe Gly Arg Asn Asp Thr Cys Asp
 580 585 590
 Cys Glu Ile Asp Asp Asp Arg Leu Ser Lys Leu His Cys Val Ile Thr
 595 600 605
 Lys Glu Asn Asp Ser Ile Trp Leu Leu Asp Lys Ser Thr Asn Ser Cys
 610 615 620
 Leu Val Asn Asn Thr Ser Val Gly Lys Gly Asn Lys Val Leu Leu Arg
 625 630 635 640
 Gly Gly Glu Ile Leu His Leu Phe Phe Asp Pro Leu Ser Ser Gln His
 645 650 655
 Ile Gly Phe Lys Val Val Leu Val Asp Gln Ser Ser Gly Glu His Lys
 660 665 670
 Ser Gln Val Glu Val Leu Lys Gln Thr Ser Glu Glu Met Asn Ile Ile
 675 680 685

Pro Leu Ile Ser Gly Leu Ser Ser Ile Ser Ser
690 695

<210> 13

<211> 295

<212> PRT

<213> Candida albicans

<400> 13

Met Gly Thr Ser Thr Ser Glu Ala Leu Lys Asn Ile Lys Asn Lys Gln
1 5 10 15

Arg Arg Gln Lys Val Phe Ala Glu Ile Lys His Glu Lys Asn Lys Gln
20 25 30

Arg His Lys Gln Arg Ala Glu Arg Ala Lys Glu Glu Arg Glu Asn Pro
35 40 45

Glu Leu Arg Glu Glu Arg Ile Ala Ala Asn Ile Pro Asp Thr Ile Asp
50 55 60

Ser Lys Arg Ile Tyr Asp Glu Thr Ile Ala Ala Glu Val Glu Gly Asp
65 70 75 80

Asp Glu Phe Gln Ser Tyr Phe Thr Asn Leu Leu Glu Glu Pro Lys Ile
85 90 95

Leu Leu Thr Thr Ser Ala Asn Ala Lys Lys Pro Ala Tyr Glu Phe Ala
100 105 110

Asp Met Ile Met Asp Phe Leu Pro Asn Val Thr Phe Ile Lys Arg Lys
115 120 125

Lys Glu Tyr Thr Met Gln Asp Met Ala Lys Tyr Cys Ser Asn Arg Asp
130 135 140

Phe Thr Ala Leu Leu Val Ile Asn Glu Asp Lys Lys Lys Val Asn Gly
145 150 155 160

Ile Thr Leu Ile Asn Leu Pro Glu Gly Pro Thr Phe Tyr Phe Ser Ile
165 170 175

Thr Ser Ile Val Asp Gly Lys Arg Ile Lys Gly His Gly Lys Ala Gly
180 185 190

Asp Tyr Leu Pro Glu Ile Val Leu Asn Asn Phe Asn Ser Arg Leu Gly

195	200	205
Lys Thr Val Gly Arg Leu Phe Gln Ser Ile Phe Pro His Lys Pro Glu		
210	215	220
Leu Gln Gly Arg Gln Val Ile Thr Leu His Asn Gln Arg Asp Tyr Ile		
225	230	235 240
Phe Phe Arg Arg His Arg Tyr Ile Phe Arg Asn Glu Glu Lys Val Gly		
245	250	255
Leu Gln Glu Gly Pro Gln Phe Thr Leu Lys Leu Arg Arg Met Gln Lys		
260	265	270
Gly Val Arg Gly Asp Val Val Trp Glu His Arg Pro Asp Met Glu Arg		
275	280	285
Asp Lys Lys Lys Phe Tyr Leu		
290	295	

<210> 14
 <211> 55
 <212> PRT
 <213> Candida albicans

<400> 14
 Met Asn Ser Glu Lys Ile Ile Glu Val Ile Ile Ala Ile Phe Leu Pro
 1 5 10 15
 Pro Val Ala Val Phe Met Lys Cys Gly Ala Thr Thr Pro Leu Trp Ile
 20 25 30
 Asn Leu Val Leu Cys Ile Phe Ile Trp Phe Pro Ala Ile Leu His Ala
 35 40 45
 Leu Tyr Val Val Leu Lys Asp
 50 55

<210> 15
 <211> 773
 <212> PRT
 <213> Candida albicans

<400> 15
 Met Thr Leu Gly Phe Asp Lys Phe Ile Ser Lys Val Ser Thr His Arg
 1 5 10 15

Arg Gln Ser Glu Pro Ser Ile Leu Glu Ile Ala Ala Thr Asn Ser Gln
 20 25 30

Asn Lys Ser Arg Arg Leu Ser Met Asp Asn Gly His Cys Tyr Val Arg
 35 40 45

Glu Ser Thr Asn Asn His His His Leu Asn Thr Val Val Asp Asn Leu
 50 55 60

Arg Gln Arg Ala Gly Ser Phe Ser Phe Ile Ser His His His Asn His
 65 70 75 80

His Gln Asn Ser His Asp Asn Tyr Thr Val Asp Pro Leu Thr Ser Asn
 85 90 95

Gly Ala Arg Ile Ser Arg Ser Arg Ser Arg Ser Lys Ser Val Gly His
 100 105 110

Gly Glu Ala Ile Ser Pro Ala Tyr Phe Ser Lys Asn Lys Thr Lys Asp
 115 120 125

Leu Val Lys Gln Glu Thr Ala His Ile Ile Ser Lys Lys Leu Leu Asn
 130 135 140

Met Leu Gln Asp Leu Asp Leu Gln Asn Pro Ile Ala Leu Lys Thr Ile
 145 150 155 160

Ser Gln Gly Ser Glu Ser Lys Phe Cys Lys Ile Tyr Val Ser Asn Thr
 165 170 175

Asn Asn Cys Ile Tyr Leu Pro Ala Ala Ser Ser Thr Ser Phe Thr Tyr
 180 185 190

Glu Asp Asp Glu Asn Gly Gly Val Ile Ile Ala Glu Asp Arg Asn Asp
 195 200 205

Glu Met Pro Thr Ala Val Asn Asn Asn Thr Leu Ser Met Asp Ser Ile
 210 215 220

Asn His Ser Glu Thr Asp Phe Ser Asp Ser Pro Pro Pro Pro Asp Leu
 225 230 235 240

Phe Ser Lys Met Lys Ser Phe His Ser Pro Asn Tyr Leu Thr Ser Lys
 245 250 255

Ile Asp Ser Glu Cys Pro Ile Pro His Thr Phe Ala Val Ile Val Glu
 260 265 270

Leu Thr Lys Asp Ser Leu Ile Ile Lys Asp Leu His Phe Gln Phe Gln
 275 280 285

Ser Leu Thr Thr Ile Leu Trp Pro Thr Gly Asp Ala Tyr Asn Arg Thr
 290 295 300

His Ala Lys Glu Lys Phe Thr Ile Gly Asn Met Glu Trp Arg Thr Ser
 305 310 315 320

Leu Ser Asp Ala Asp Tyr Tyr Ile Asn Ser Ser Asn Ser Asn Asp Val
 325 330 335

Lys Ser Lys Asn Leu Gly Pro Glu Asp Leu Ile Asn Arg Thr Arg Glu
 340 345 350

Tyr Lys Leu Ile Asp Ile Glu Glu Pro Asn Asn Ser Ser Asn Ser Leu
 355 360 365

Ser Asp Asp Asp Met Asp Ile Asn Asn Ile Thr Ser Pro Leu Ser Thr
 370 375 380

Ser Pro Thr Ser Ser Ser Thr Ser Thr Asn Ser Thr Ser Asn Ser Leu
 385 390 395 400

Gly Ser Asp Ser Tyr Lys Ala Gly Leu Tyr Val Phe Leu Leu Pro Ile
 405 410 415

Leu Leu Pro Glu His Ile Pro Ala Ser Ile Val Ser Ile Asn Gly Ser
 420 425 430

Leu Ala His Thr Leu Ser Val Glu Cys Asn Lys Tyr Thr Asp Lys Leu
 435 440 445

Asn Arg Lys Ser Lys Val Ser Ala Ser Tyr Asn Leu Pro Met Val Arg
 450 455 460

Thr Pro Pro Asn Ile Gly Asn Ser Ile Ala Asp Lys Pro Ile Tyr Val
 465 470 475 480

Asn Arg Ile Trp Asn Asp Ala Val His Tyr Ile Ile Thr Phe Pro Arg
 485 490 495

Lys Tyr Val Thr Leu Gly Cys Glu His Met Ile Asn Val Lys Leu Ser
 500 505 510

Pro Met Val Lys Asp Val Val Ile Lys Arg Ile Lys Phe Asn Val Leu
 515 520 525

Glu Arg Ile Thr Tyr Val Ser Lys Asn Leu Ser Arg Glu Tyr Asp Tyr
 530 535 540

Asp Ser Glu Asp Pro Tyr Cys Ile His Pro Val Ser Lys Glu Asn Lys
 545 550 555 560

Val Arg Glu Arg Val Val Ser Leu Tyr Glu Leu Lys Thr Lys Ala Lys
 565 570 575

Gln Ser Ser Gly Gly His Leu Glu Ala Tyr Lys Gln Glu Val Met Lys
 580 585 590

Cys Pro Glu Asn Asn Leu Leu Phe Ser Cys Tyr Glu Val Glu Asn Asp
 595 600 605

Asn Asn Asn Gly Asn Gly Asn Gly Asn Gly Asn Gly Asn Lys Asn Val
 610 615 620

Lys Gln Lys Asn Lys Asp Gln Pro Met Ile Ala Thr Pro Leu Asp Ile
 625 630 635 640

Asn Val Ser Leu Pro Phe Leu Thr Thr Met Ser Asp Ser Leu Ile Met
 645 650 655

Thr Ser Ala Ile Glu Glu Glu Gly Ser Asp Ser Pro His Thr Ser Arg
 660 665 670

Arg Gly Ser Ala Val Ser Met Thr Asp Asn Asn Thr Thr Pro Ser Asn
 675 680 685

Asn Asn Pro Leu Ser Pro Phe Leu Gly Ala Val Glu Thr Asn Gly Ala
 690 695 700

Ser Ile Asn Glu Ile Gly Asp His Thr Leu Phe Pro Asp Ser Asn Phe
 705 710 715 720

Arg His Ile Glu Ile Lys His Arg Leu Gln Val Thr Phe Arg Ile Ser
 725 730 735

Lys Pro Asp Ser Asp Asn Lys Met His His Tyr Glu Val Val Ile Asp
 740 745 750

Thr Pro Ile Val Leu Leu Ser Ser Lys Cys Gln Glu Asp Ser Pro Pro
 755 760 765

Pro Tyr Ser Ser Val
 770

<210> 16

<211> 90

<212> PRT

<213> Candida albicans

<400> 16

Met Gly Glu Gly Thr Pro Ser Leu Gly Lys Arg His Asn Lys Ser His
 1 5 10 15

Thr Leu Cys Asn Arg Cys Gly Arg Arg Ser Phe His Val Gln Lys Lys
 20 25 30

Thr Cys Ser Ser Cys Gly Tyr Pro Ala Ala Lys Met Arg Ser His Asn
 35 40 45

Trp Ala Leu Lys Ala Lys Arg Arg Arg Thr Thr Gly Thr Gly Arg Met
 50 55 60

Ala Tyr Leu Lys His Val Thr Arg Arg Phe Lys Asn Gly Phe Gln Thr
 65 70 75 80

Gly Val Ala Lys Ala Gln Thr Pro Ser Ala
 85 90